# THE PLANT DISEASE REPORTER

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Growth Through Agricultural Progress

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Paul R. Miller

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Please note revisions in "Suggestions for Preparation of Manuscripts."

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# CHITIN AND THE BIOLOGICAL CONTROL OF FUSARIUM DISEASES[1]

R. Mitchell and M. Alexander[2]

Summary

The addition of small quantities of chitin to soil resulted in a marked reduction in the severity of root-rot of beans caused by Fusarium solani f. phaseoli. Chitin amendment was also effective in reducing vascular wilt of radishes caused by Fusarium oxysporum f. conglutinans. Various other carbon and nitrogen sources as well as chitin degradation products gave little or no reduction in the root-rot of beans.

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The biological control of soil-borne pathogens by the inoculation of antagonistic microorganisms into soil has been frequently attempted in recent years, but the results have been negative or, at best, conflicting. For example, Novogrudski (5) reported that the wilt of flax was prevented as a result of the introduction into soil of organisms known to suppress Fusarium oxysporum f. lini in culture medium. On the other hand, no control of banana wilt was noted following the inoculation of actinomycetes antagonistic to Fusarium oxysporum f. cubense (3).

In view of the problems inherent to the establishment of a new population in a natural microbial environment, in which the composition of the microflora is governed by the already existing biological interactions, the addition of crop residues or other carbonaceous materials to stimulate a flora detrimental to the pathogen seems to be a more promising approach to biological control of plant pathogens. Thus, damping off is reduced by the incorporation of wheat straw (1), and barley straw and other organic materials reduce the severity of a Rhizoctonia solani disease of snap beans (2).

In the present study, the effect of soil amendment with chitin on Fusarium diseases of radish and bean was determined, and the degree of disease control obtained with chitin was compared with that obtained with other amendments. The results suggest that specific substrates for the microflora may stimulate selectively a segment of the population that suppresses, directly or indirectly, the soil-borne pathogens.

## MATERIALS AND METHODS

The soils used in this study were the Honeoye silt loam, a well drained soil of pH 7.3, and the Williamson silt loam, a moderately well drained acid soil of pH 5.6. The field from which the latter soil had been taken was heavily infested with Fusarium solani f. phaseoli for some 40 years. The Williamson soil was mixed with vermiculite in a 1:1 ratio prior to use in the greenhouse. In investigations with Honeoye silt loam, three replicate flats containing a total of 60 bean plants or 240 radish plants were used for each treatment. With the Williamson silt loam, four replicate pots containing a total of 16 bean plants were used for each treatment. Red kidney bean (Phaseolus vulgaris) was the host in the study of bean root-rot, and the Bountiful variety of radish was used for vascular wilt studies.

Direct inoculation of bacteria onto seed was achieved by means of Nitra-coat, a seed coating preparation obtained from the Nitragin Company, Milwaukee, Wisconsin. The chitin and a deacetylated derivative of the aminopolysaccharide, chitosan, were obtained from the Moretex Chemical Products, Spartanburg, South Carolina. The chitin was finely ground and mixed with the soil to a depth of 6 inches. The culture of F. solani f. phaseoli was provided by Dr. D. F. Bateman, Department of Plant Pathology, Cornell University.

A disease index was established as a measure of the severity of root-rot or vascular wilt. A zero rating reflected no disease, and a value of 100 indicated that the plant was killed. Intermediate ratings of 20, 40, 60 and 80 were used to indicate different grades of disease, and the index was calculated for each replicate by averaging the sum of the values for each plant.

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[1]Supported in part by a grant from the United Fruit Company, Boston, Massachusetts. Agronomy Paper No. 536.

[2]Research Assistant and Associate Professor of Soil Science, Cornell University.

## RESULTS

Strains of Bacillus and Pseudomonas capable of lysing F. oxysporum f. cubense were previously isolated from a number of soils (4). Initial attempts to control the root-rot of beans caused by F. solani f. phaseoli either by the direct addition of the lytic species to soil or by the coating of seeds of susceptible plants with the active bacteria proved to be totally ineffective as a means of disease control.

Because all the lytic bacteria were capable of utilizing chitin, a characteristic not too widespread among soil bacteria, this polysaccharide was added to infested soil to stimulate chitinase-producing microorganisms, some of which might be able to lyse and destroy the pathogen. The data of Table 1 demonstrate that chitin added at a rate of 200 pounds/acre markedly reduced the severity of the rot in infested soil. In this and subsequent tables the brackets are used to reflect significant differences at the 5% level among the various means as measured by the Duncan multiple range test. Means enclosed by the same brackets are not significantly different from one another; those not so enclosed are significantly different. Neither cellulose nor ammonium chloride had any statistically significant effect on the disease severity.

In a similar investigation designed to ascertain the influence of this amendment on radish wilt, again chitin at application rates of 200 pounds/acre reduced appreciably the severity of disease (Table 2). Inorganic nitrogen was again without effect, but cellulose with or without the ammonium salt in this instance apparently aggravated the wilt.

More detailed studies were carried out with F. solani f. phaseoli in Williamson silt loam, a soil which had been heavily infested with that pathogen for many years. The effect of different quantities of chitin on the root-rot is shown in Table 3. In this heavily but naturally infested environment, the incorporation of 200 pounds/acre of chitin appeared to have a marked influence, but the reduction in disease index was not statistically significant at the 5% level. A two-fold increase in application rate, however, gave a significant reduction. Undoubtedly, the lower rate would have been significant were additional replicates included.

A comparison was made between the use of chitin mixed throughout the surface 6 inches of soil and a concentrated banding of the polysaccharide directly below the seed. No differences in disease control were observed between the two treatments.

To determine whether chitin was selective in its action or whether the choice was fortuitous, a number of different carbon sources were applied to soil. Ammonium chloride was added together with those amendments containing no nitrogen. Agar is a galactose polymer, pectin is a polysaccharide of galacturonic acid units while glucose is the monomer of starch and of the cellulose previously used. Glucose and N-acetylglucosamine are products of chitin decomposition. None of these compounds reduced the severity of disease to the same degree as chitin (Table 4). Some control was noted with pectin, however. In a separate study in which chitin and chitosan, another degradation product of the polymer, were added to soil at a rate of 400 pounds/acre, the disease indices for chitin, chitosan and untreated control were 46, 70 and 79, respectively. The effect of chitin was statistically significant whereas there was no significant effect resulting from chitosan incorporation. Hence, of the compounds examined to date, chitin appears to be unique.

## DISCUSSION

The concept of biological disease control is based on the assumption that a pathogen can be suppressed by saprophytic microorganisms initially present in or artificially added to the environment. In the current investigation, direct inoculation into soil of bacteria known to have a deleterious effect on fusaria resulted in no significant reduction in severity of disease. Such failures are not surprising since the indigenous population reflects the physical and chemical constitution of the habitat, and the mere addition of organisms, be they native or alien, should not alter appreciably the established population equilibrium. The inability to eradicate fungi susceptible to digestion by the inoculum of lytic bacteria probably can be attributed to the anticipated decline and disappearance of the inoculum.

A more promising method of disease control seems to be through the use of substrates that selectively stimulate that segment of the indigenous population that acts to the detriment of the pathogen. Since all of the lytic bacteria were capable of producing chitinase, it seemed reasonable to assume that at least some of the chitinase-producing microorganisms of soil were lytic to the fusaria, and the fact that additions of relatively small quantities of chitin gave significant disease control suggested that the activities of lytic bacteria were indeed implicated

Table 1. Use of chitin to control root-rot of kidney beans in Honeoye silt loam.

| Amendment | Disease index |
|---|---|
| None | 40 |
| Cellulose, 110 pounds/acre | 44 |
| Ammonium chloride, 90 pounds/acre | 42 |
| Cellulose + ammonium chloride | 34 |
| Chitin, 200 pounds/acre | 19 |
| Unamended, uninoculated control[a] | 0 |

[a]No pathogen added. In all other treatments *F. solani* f. *phaseoli* was added to the soil.

Table 2. Control of vascular wilt of radishes by addition of chitin to Honeoye silt loam.

| Amendment | Disease index |
|---|---|
| Cellulose + ammonium chloride | 86 |
| Cellulose, 110 pounds/acre | 71 |
| Ammonium chloride, 90 pounds/acre | 48 |
| None | 46 |
| Chitin, 200 pounds/acre | 10 |
| Uninoculated, unamended control[a] | 10 |

[a]No pathogen added. In all other treatments *F. oxysporum* f. *conglutinans* was added to the soil.

Table 3. Control of bean root-rot by different quantities of chitin in Williamson silt loam.

| Chitin amendment (pounds/acre) | Disease index |
|---|---|
| 0 | 77 |
| 200 | 52 |
| 400 | 45 |
| 800 | 48 |
| 1200 | 18 |

Table 4. Effect of various amendments on the severity of bean root-rot in Williamson silt loam.

| Treatment[a] | Disease index |
|---|---|
| Starch + ammonium chloride | 74 |
| None | 70 |
| Glucose + ammonium chloride | 67 |
| N-acetylglucosamine | 66 |
| Agar + ammonium chloride | 63 |
| Pectin + ammonium chloride | 54 |
| Chitin | 32 |

[a]Starch, glucose, agar and pectin were each added at a rate equivalent to 450 pounds/acre, chitin and N-acetylglucosamine at 500 pounds/acre and $NH_4Cl$ at 120 pounds/acre.

in the disease control. Chitin, however, is known to stimulate the actinomycete population so that an alternative explanation of the chitin effect involves the production by actinomycetes of substances active against the fungi. Although evidence for a major ecological role for antibiotics in soil is far from unequivocal, the correlation between the reduction in disease index and the stimulation of actinomycetes by chitin amendment may be an indication that a means has been obtained of specifically selecting antibiotic-synthesizing species.

Hence, whether chitin be a practical soil amendment or not, it seems clear that certain carbonaceous nutrients when added to soil at reasonable application rates may serve to initiate the development of a population active in the control of soil-borne pathogens. Considerable further study is required, however, to determine the most suitable substrates and the mechanism of their action.

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# PLANT PARASITIC NEMATODES ASSOCIATED WITH DECLINE OF WOODY ORNAMENTALS IN NORTH CAROLINA AND THEIR CONTROL BY SOIL TREATMENT

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Summary

Fourteen genera of plant-parasitic nematodes were found associated with six kinds of woody ornamentals in varying stages of decline in North Carolina during 1959 and 1960. Frequency of occurrence and relative population levels of the various forms give an indication of their involvement as pathogens of these hosts. Effective control of all but the stubby-root nematode (Trichodorus spp.) was achieved by injecting 1,2-dibromo-3-chloropropane in an area approximately 3 feet in diameter around established plants at dosages ranging from 4 to 10 gallons/acre. Dosage response was related to soil type. Favorable response of treated plants was noticeable usually in 8 to 10 weeks following treatment.

Less than three decades ago decline of woody ornamentals in the southeastern United States was attributed largely to an unfavorable environment involving either infertile soil, extreme moisture fluctuations, extremes of temperature, or combinations of these. In many instances unseasonable climatic stresses, such as early fall or late spring freezes, extended drought, or extended periods of heavy precipitation, have caused great damage. More recently, however, it has been shown that decline of woody ornamentals often is caused by parasitic nematodes. The damage wrought by these parasites is characterized by poor growth, low vigor, yellowing or bronzing of foliage, dieback of branches, restricted and distorted root system, and in many instances death of the plant; and this can occur under climatic conditions favorable for the plant. When plants weakened by nematodes are subjected to climatic stresses, the rapidity of decline is usually accelerated.

The development of effective nematocides during the past two decades and particularly the more recent advent of selective nematocides that can be applied to the living plant without harmful effects has proved a boon to the commercial nurseryman, the landscape architect, and the home owner.

This paper reports 1) the results of a survey made during 1959 and 1960 to determine the kinds of parasitic nematodes associated with some of the more popular woody ornamentals grown in North Carolina and the frequency of their occurrence and relative population level, and 2) the effects of treatment of nursery plants with different rates of 1,2-dibromo-3-chloropropane[1] (DBCP) at different seasons of the year and on different soil types upon plant-parasitic nematode populations and plant growth response.

## METHODS

The survey included 86 locations and 6 kinds of ornamental shrubs and represented problem situations where the involvement of parasitic nematodes was suspected. A wide variety of sites and soil types was represented. Moreover, the plants were of various sizes; some were growing in nurseries, others in home plantings.

Data were obtained from laboratory assays of soil samples taken from the root zone of plants in various stages of decline. In most cases each sample was comprised of several cores of soil taken by means of a sampling tube and contained root fragments. Samples were generally processed within 48 hours after they were taken. Separation of nematodes from the samples was accomplished by a modified Baermann funnel technique[2]. Although this procedure has proved satisfactory for most kinds of nematodes encountered, there is evidence that it frequently gives relatively low estimates of populations of ring nematodes and perhaps other sluggish forms.

Fumigation tests were conducted in naturally infested sites, hence, several genera of parasitic nematodes were usually present in any given location. A pre-treatment census of nematode populations was made for purposes of site selection. Tests were conducted at sites where 1) population density of one or more species of parasitic nematodes was 500 or more

[1]The writers are indebted to Mr. Gus Kirchhof, representative of the Shell Chemical Company, for technical assistance in the field and to the Shell Chemical Company for supplying Nemagon EC-2, the formulation of 1,2-dibromo-3-chloropropane used in this study.

[2]Perry, J. L. 1956. Laboratory assays of soil samples for plant-parasitic nematodes. M.S. Thesis, North Carolina State College.

specimens per pint of soil, and 2) where an ample supply of infested plant material was available for treatment and observation.

Application of DBCP was made by injecting appropriate amounts of the material at intervals of approximately 12 inches and at depths of 6 to 8 inches. In general, treatments were applied to an area approximately 3 feet in diameter around established plants. Soil temperatures at 6- to 8-inch depth ranged from 58° to 72° F; soil moisture levels were in a favorable range for fumigation treatment.

Treatment effects were measured by assaying nematode populations at intervals after treatment and by observations upon plant growth.

## RESULTS AND DISCUSSION

Survey: Fourteen genera of plant-parasitic nematodes were found in survey samples (Table 1). In many cases specimens were identified to species.

Although the frequency of occurrence and population density of the various forms may give some indication of their importance to decline, proof of pathogenicity has been established in only a few cases. Moreover, the population level is not always correlated with the extent of damage. The existence of very high levels may, in fact, indicate the ability of the host to support such populations; whereas a low level may be found where the parasite has inflicted severe damage. No doubt some of the forms encountered in certain situations were not involved in decline but their presence makes them suspects until more specific information is acquired. An interpretation of the survey results upon each host follows.

1. Azalea. (Rhododendron spp.). Moderate to high populations of the stunt nematode, Tylenchorhynchus claytoni, were found in a large proportion of the samples. Azalea appears to be a suitable host for this species that may be responsible for decline. Next in prevalence were spiral nematodes, Helicotylenchus erythrinae and H. nannus, the dagger nematode, Xiphinema americanum, the lance nematode, Hoplolaimus tylenchiformis, stubby-root nematodes, Trichodorus spp., ring nematodes, Criconemoides spp., and sheath nematodes, Hemicycliophora spp. Although root-knot nematode larvae, Meloidogyne spp., were found in six samples, populations were relatively low.

2. Boxwoods. (Buxus sempervirens arborescens and B. s. suffruticosa). Lesion nematodes, Pratylenchus spp., and spiral nematodes, Helicotylenchus spp., are known to cause decline in boxwoods. In the Piedmont area P. vulnus was the species most commonly encountered; whereas, P. penetrans and P. zeae were found frequently in the coastal plains. H. buxophilus was the most prevalent spiral nematode although H. erythrinae and H. nannus also were found. A species of Scutellonema was found at one location. Dagger, ring and stubby-

FIGURE 1. Natural infection of the root-knot nematode, Meloidogyne hapla, in liners of Ilex crenata.

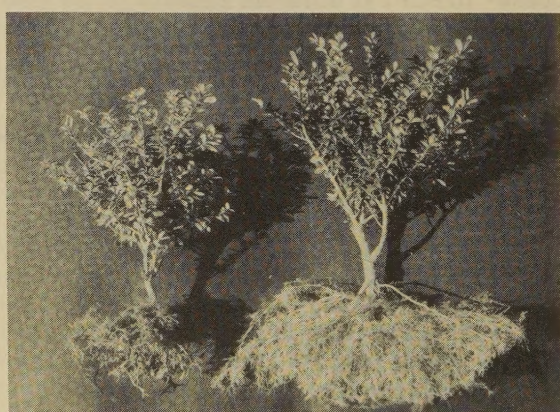

FIGURE 2. Response of Japanese holly (Ilex crenata) to treatment with DBCP at the rate of 6 gallons per acre. Nontreated check plant at left is severely damaged by the root-knot nematode, Meloidogyne incognita. Treated plant at right is healthy. Planting was established in the spring of 1955. Treatment was applied in October 1956. Plants were dug and photographed in December 1958.

Table 1. Frequency of occurrence and population levels of plant parasitic nematodes associated with decline of six kinds of ornamental shrubs in North Carolina, 1959-60.

| Host plant | No. of locations | No. of samples | Nematodes found | Frequency of occurrence | Nematodes per pint of soil: average | Nematodes per pint of soil: maximum |
|---|---|---|---|---|---|---|
| Azalea | 24 | 47 | stunt | 37 | 1095 | 6425 |
| | | | spiral | 15 | 316 | 1500 |
| | | | dagger | 11 | 550 | 1550 |
| | | | lance | 9 | 417 | 2025 |
| | | | stubby-root | 9 | 193 | 425 |
| | | | ring | 8 | 95 | 450 |
| | | | root-knot | 6 | 157 | 575 |
| | | | sheath | 5 | 90 | 150 |
| | | | lesion | 1 | 475 | 475 |
| | | | awl | 1 | 175 | 175 |
| Boxwood (American) | 20 | 77 | lesion | 55 | 1025 | 4200 |
| | | | dagger | 50 | 117 | 1650 |
| | | | stubby-root | 41 | 446 | 1450 |
| | | | ring | 33 | 103 | 2675 |
| | | | spiral | 28 | 208 | 4550 |
| | | | stunt | 15 | 40 | 544 |
| | | | lance | 6 | 157 | 512 |
| | | | sheath | 5 | 140 | 350 |
| | | | root-knot | 3 | 110 | 175 |
| | | | needle | 2 | 11 | 32 |
| | | | pin | 1 | 216 | 216 |
| | | | sting | 1 | 16 | 16 |
| Boxwood (English) | 15 | 133 | lesion | 105 | 1499 | 4265 |
| | | | spiral | 95 | 898 | 9150 |
| | | | root-knot | 16 | 195 | 525 |
| | | | stubby-root | 11 | 185 | 600 |
| | | | ring | 11 | 98 | 375 |
| | | | dagger | 10 | 40 | 100 |
| | | | stunt | 9 | 43 | 275 |
| | | | pin | 9 | 275 | 575 |
| | | | lance | 5 | 245 | 850 |
| | | | sheath | 1 | 126 | 126 |
| Camellia | 13 | 31 | lance | 16 | 101 | 450 |
| | | | dagger | 16 | 154 | 2050 |
| | | | stunt | 12 | 779 | 4100 |
| | | | stubby-root | 12 | 108 | 225 |
| | | | spiral | 11 | 1002 | 5825 |
| | | | lesion | 10 | 55 | 225 |
| | | | sheath | 8 | 1871 | 15250 |
| | | | ring | 6 | 230 | 1650 |
| | | | needle | 4 | 13 | 50 |
| | | | root-knot | 1 | 25 | 25 |
| Holly | 11 | 40 | dagger | 26 | 433 | 3860 |
| | | | stubby-root | 16 | 172 | 550 |
| | | | spiral | 11 | 648 | 2464 |
| | | | stunt | 10 | 80 | 175 |
| | | | root-knot | 5 | 4180 | 15050 |
| | | | lance | 4 | 308 | 650 |
| | | | lesion | 3 | 350 | 850 |
| | | | sheath | 2 | 375 | 650 |
| | | | pin | 2 | 812 | 900 |
| | | | ring | 1 | 750 | 750 |
| Rose | 3 | 7 | spiral | 4 | 450 | 1550 |
| | | | lesion | 4 | 625 | 1025 |
| | | | stunt | 3 | 233 | 275 |
| | | | dagger | 1 | 425 | 425 |
| | | | root-knot | 1 | 375 | 375 |
| | | | stubby-root | 1 | 16 | 16 |

Table 2. The effect of DBCP injections in the root zone of azalea plants on populations of associated parasitic nematodes and as influenced by dosage, season, and soil type.

| Locality and soil type | Dosage per acre (in gallons) | Nematode species | Nematodes per pint of soil: before treatment | Nematodes per pint of soil: after treatment |
|---|---|---|---|---|
| | | | June 23, 1960 | Sept. 30, 1960 |
| Raleigh, N. C. Wake Co. (Cecil sandy loam modified with incorporation of peat moss and compost) | 6 | stunt | 957 | 0 |
| | | stubby-root | 75 | 0 |
| | | spiral | 56 | 0 |
| | | sheath | 28 | 0 |
| | | dagger | 16 | 5 |
| | | lance | 397 | 20 |
| | | ring | 56 | 0 |
| | none | stunt | 0 | 425 |
| | | lance | 2025 | 550 |
| | | | May 30, 1960 | Sept. 30, 1960 |
| Jacksonville Onslow Co. (Norfolk sandy loam) | 6 | stunt | 825 | 0 |
| | | dagger | 1550 | 0 |
| | | ring | 75 | 0 |

Table 3. The effect of DBCP injections in the root zone of American boxwood plants on populations of associated parasitic nematodes and as influenced by dosage, season, and soil type.

| Locality and soil type | Dosage per acre (in gallons) | Nematode species | Nematodes per pint of soil: before treatment | Nematodes per pint of soil: after treatment |
|---|---|---|---|---|
| | | | July 14, 1960 | Oct. 21, 1960 |
| John's River (Hiawassee clay) | 6 | lesion | 1030 | 225 |
| | | spiral | 150 | 150 |
| | | dagger | 200 | 25 |
| | | stubby-root | 460 | 2875 |
| | | ring | 60 | 115 |
| | 8 | lesion | 1200 | 90 |
| | | spiral | 125 | 10 |
| | | dagger | 200 | 0 |
| | | stubby-root | 625 | 125 |
| | | ring | 100 | 0 |
| | none | lesion | 1010 | 2220 |
| | | spiral | 125 | 250 |
| | | dagger | 150 | 125 |
| | | stubby-root | 550 | 500 |
| | | ring | 150 | 75 |
| | | | June 13, 1960 | Nov. 3, 1960 |
| Erwin Harnett Co. (Norfolk sandy loam) | 6 | lesion | 125 | 0 |
| | | spiral | 1450 | 0 |
| | | sheath | 275 | 0 |
| | | | July 23, 1959 | Sept. 29, 1960 |
| Rockingham (Norfolk sandy loam) | 6 | lesion | 575 | 0 |
| | | lance | 80 | 0 |
| | | sheath | 25 | 0 |
| | | needle | 25 | 0 |
| | | stunt | 0 | 25 |
| | | dagger | 0 | 12 |
| | none | lesion | 625 | 465 |
| | | spiral | 187 | 125 |
| | | sheath | 12 | 25 |
| | | lance | 25 | 112 |

Table 4. The effect of DBCP injections in the root zone of English boxwood plants on populations of associated parasitic nematodes and as influenced by dosage, season, and soil type.

| Locality and soil type | Dosage per acre (in gallons) | Nematode species | Nematodes per pint of soil: before treatment | Nematodes per pint of soil: after treatment | |
|---|---|---|---|---|---|
| | | | May 9, 1960 | Aug. 5, 1960 | Oct. 21, 1960 |
| | 4 | lesion | 180 | 0 | 105 |
| | | spiral | 210 | 0 | 25 |
| | 6 | lesion | 600 | 0 | 95 |
| | | spiral | 2180 | 0 | 30 |
| Mount Airy Surry Co. (Cecil clay) | 8 | lesion | 430 | 25 | 5 |
| | | spiral | 2065 | 0 | 0 |
| | 10 | lesion | 130 | 0 | 25 |
| | | spiral | 645 | 0 | 0 |
| | none | lesion | 451 | 336 | 2246 |
| | | spiral | 1043 | 1480 | 2000 |
| | | | Oct. 29, 1959 | Feb. 10 1960 | Sept. 19 1960 |
| Fuquay Wake Co. (Norfolk sandy loam) | 6 | lesion | 4265 | 275 | 0 |
| | | spiral | 812 | 32 | 0 |
| | | lance | 96 | 0 | 0 |
| | | dagger | 0 | 25 | 0 |
| | | stubby-root | 0 | 50 | 275 |

Table 5. The effect of DBCP injections in the root zone of Japanese holly plants on populations of associated parasitic nematodes and as influenced by dosage, season, and soil type.

| Locality and soil type | Dosage per acre (in gallons) | Nematode species | Nematodes per pint of soil: before treatment | Nematodes per pint of soil: after treatment | |
|---|---|---|---|---|---|
| | | | Sept. 13, 1959 | July 26, 1960 | |
| Raleigh Wake Co. (Cecil loam) | 4 | lesion | 875 | 0 | |
| | | spiral | 1850 | 800 | |
| | | dagger | 750 | 0 | |
| | | stubby-root | 0 | 350 | |
| | | | April 12, 1959 | Aug. 19, 1959 | Apr. 6, 1960 |
| Greenville Pitt Co. (Norfolk sandy loam) | 6 | root-knot | 2275 | 0 | 0 |
| | | lesion | 25 | 0 | 0 |
| | | stunt | 75 | 0 | 0 |
| | | spiral | 50 | 0 | 0 |
| | | stubby-root | 0 | 283 | 150 |
| | | dagger | 25 | 0 | 0 |

root nematodes are considered as possible parasites of damaging proportions to American boxwood, whereas root-knot and lance nematodes are probable pathogens of English boxwood.

3. *Camellia*. (*C. japonica* and *C. sasanqua*). Sheath nematodes, *Hemicycliophora* spp., spiral nematodes, stunt nematodes, dagger nematodes and ring nematodes are probable parasites of camellia. The root-knot nematodes, *Meloidogyne incognita* and *M. javanica*, are parasitic on some varieties but were of no consequence in the areas sampled.

4. *Holly*. (*Ilex crenata*). Ten genera of plant-parasitic nematodes were found associated with Japanese holly. The root-knot nematodes, *Meloidogyne* spp., undoubtedly comprise the most important group (Fig. 1). Several other species, particularly those found in several samples, are considered as probable parasites of this crop. This includes the dagger, stubby-root, spiral, stunt, lance and lesion nematodes.

5. *Rose*. (*Rosa multiflora*). Only a limited number of samples from rose was studied. Spiral and lesion nematodes are regarded as probable parasites. Root-knot nematodes are known to attack rose but only one case was encountered.

Soil Treatments: Nematode population data from experiments with azalea are given in Table 2. In sandy soils, injections of DBCP at a rate of 6 gallons per acre practically eliminated stunt and lance nematodes at one location and reduced stunt and dagger nematodes to below detectable levels at a second location.

American boxwood, growing in clay soil (Hiawassee), was heavily infested with lesion nematodes and to a lesser extent with spiral, dagger and stubby-root nematodes. An injection of DBCP at 6 gallons per acre was not effective. Application of 8 gallons per acre reduced populations to low levels (Table 3). In porous soil an injection of 6 gallons of DBCP per acre reduced lesion and spiral nematodes to non-detectable levels (Table 3).

English boxwood plants growing in a compact clay soil (Cecil) were treated with DBCP at 4, 6, 8, and 10 gallons per acre. Ninety days after treatment practically no nematodes were encountered; but, after 150 days the nematodes were held to a minimum only in plots receiving the higher rates of the nematocide, namely 8 and 10 gallons per acre (Table 4). In a sandy loam soil (Norfolk) nematode populations were greatly diminished 100 days after a fall treatment with DBCP at the rate of 6 gallons per acre. Stubby-root nematodes were the only ones recovered after an 11-month interval.

Injections of DBCP at 4 gallons per acre appeared to control lesion and dagger nematodes affecting Japanese holly but did not effectively control spiral nematodes (Table 5). Stubby-root nematode populations increased after treatment. In a sandy loam soil (Norfolk) high populations of root-knot nematodes were apparently eliminated with injections of DBCP at a rate of 6 gallons per acre, but stubby-root nematode populations increased (Table 5).

Response to Treatment: Experimental sites were visited at intervals following treatment with DBCP to observe the extent of recovery. Rose bushes with marked symptoms of decline and supporting high populations of lesion and spiral nematodes showed marked improvement 8 weeks after treatment at a dosage of 6 gallons per acre. Japanese holly infested with root-knot nematodes showed improvement with a similar treatment after 10 weeks and was restored to excellent vigor 4 months after treatment (Fig. 2). Azalea plants, infested with stunt and sheath nematodes made remarkable recovery 10 weeks after treatment at a dosage of 4 gallons per acre. Improved root growth of boxwood was evident 8 and 12 weeks after treatment in heavy clay soils at dosages of 6 and 8 gallons per acre.

Response to treatment varies with plant species and also with stage of decline at the time of treatment. Some plants are quite sensitive to DBCP while others are relatively tolerant of the chemical. The plants discussed in this report fall in the latter class as do most species of woody ornamentals. Plants that are normally tolerant of the chemical may, however, suffer apparent injury as a result of treatment if their root systems have been damaged severely by parasitic nematodes prior to treatment. In some instances, even though the nematode population may be greatly reduced by treatment, the plant may not respond because of excessive root destruction by the nematodes. This was observed in one instance where a few plants of Japanese holly were so heavily damaged by root-knot nematode (*M. incognita*) at the time of treatment that recovery was not achieved.

## CONCLUSIONS

1) DBCP appears to be highly toxic to root-knot, lesion, stunt, spiral, dagger, stubby-root, sheath, lance, and ring nematodes at rates of 4, 6, and 8 gallons per acre (Tables 2, 3, 4, and 5). Stubby-root nematode populations apparently build up quite rapidly after treatment (Tables 3, 4, and 5).

2) Approximately twice as much DBCP is required to control nematodes in compact soils (Cecil clay) as is required for sandy soils (Norfolk sandy loam).

3) Azaleas, American- and English-boxwood and Japanese holly tolerate injected dosages of 4 to 6 gallons per acre of DBCP in many types of soil at temperatures not exceeding 75° F. American- and English-boxwood under similar conditions tolerate concentrations up to 10 gallons per acre in compact clay soils.

4) The lethal effect of DBCP appears to continue in the soil for several months for many types of plant-parasitic nematodes. Stubby-root nematodes are an exception. Hence, it appears that shrubbery can be kept relatively free of parasitic nematodes with soil treatments spaced 20 to 24 months apart.

5) DBCP appears equally effective when injected from early April through late October provided soil temperatures are within the recommended range of 50° to 75° F and the soil is neither too wet nor too dry.

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# SWEETPOTATO PRODUCTION ON SOIL TREATED WITH SOIL FUMIGANTS

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## Abstract

Total yield of Velvet sweetpotatoes was reduced on soil treated with 1.5 gallons/acre of dibromochloropropane (DBCP), but not at dosages of 0.75, 1.0 or 1.25 gallons/acre, when applied to sandy loam soils 3 to 4 weeks prior to planting. Soil fumigation with DBCP, dibromoethane (EDB) and dichloropropene (Telone) increased yields of U. S. No. 1 roots, with the largest increases occurring on soil treated with EDB and Telone.

## INTRODUCTION

The root-knot nematode, *Meloidogyne incognita*, is a serious pest of sweetpotatoes in California. Light-textured soils are preferred for growing sweetpotatoes, and it is in these soils that the nematodes do their greatest damage. The nature of the damage and effect of nematode control on yield of sweetpotatoes in North Carolina has been described by Krusberg and Nielsen (1) and by Nielsen and Sasser (4).

EDB (1,2-dibromoethane) and D-D (1,3-dichloropropene, 1,2-dichloropropane) frequently are used for root-knot nematode control on sweetpotatoes. Martin and Pratt (2) suggest that sweetpotatoes often grow poorly following soil fumigation with a bromide-containing fumigant. McBeth and Bergeson (3) listed sweetpotato transplants as being intermediate in sensitivity to 1,2-dibromo-3-chloropropane (DBCP).

Tests were started in 1956 to determine what effect preplant soil fumigation with DBCP and other soil fumigants would have on yield and market quality of sweetpotatoes in California.

## METHODS AND MATERIALS

Three tests were conducted in commercial fields in which the variety Velvet (a selection from Puerto Rico) was grown. Data on the mechanical analysis and pH of the soils as well as temperature and moisture conditions at the time of treating are given in Table 1.

Table 1. Physical properties of fumigated soils and soil temperature and moisture at the time of treating.

| Grower | | Mechanical analysis | | | pH | Temperature | % |
|---|---|---|---|---|---|---|---|
| | | % sand | % silt | % clay | | (°F) | moisture |
| Ellis | Sandy loam | 74 | 11 | 15 | 7.6 | 65 | 9.9 |
| Scarroni | do. | 75 | 14 | 11 | 7.5 | 75 | 6.7 |
| Cacciatori | do. | 80 | 8 | 12 | 7.9 | 62 | 9.2 |

Fumigants were injected into moist well-tilled sandy loam soils with a chisel applicator at a depth of 8 inches by shanks spaced 12 inches apart. The soil surface was smoothed and cultipacked. The nematocides and the dosage applied are presented in Tables 2, 3, and 4 containing the results of the individual tests. Transplants were set approximately 1 month after treating in all tests.

## RESULTS

In the Ellis test fumigants were applied to blocks 18 feet x 200 feet replicated three times in a randomized block design. Yield of roots was obtained from two rows 100 feet long in each block. The roots were then graded by the grower and weight of roots in each grade obtained. Soil samples for determining the number of root-knot nematode larvae were not obtained, and roots in all plots were not scored directly for the presence of galls or egg masses. However, examination of feeder roots and storage roots in the nontreated plots revealed that a severe root-knot nematode infestation occurred throughout the test area. This was reflected in the reduction in the number of U. S. No. 1's from the nontreated plots and the larger number of U. S.

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Table 2. Yield and market value of sweetpotatoes in various grades. Ellis test, Loma Linda, California.

| Treatment | Dosage (gallons/acre) | U. S. No. 1 yield | U. S. No. 1 value | U. S. No. 2 yield | U. S. No. 2 value | Jumboes yield | Jumboes value | Culls yield | Total yield | Total value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Yield in pounds/acre and value in dollars[a] | | | | | | | | |
| None | --- | 3688 | 295 | 13,961 | 419 | 1902 | 76 | 3488 | 23,000 | 790 |
| DBCP | 1.5 | 7318 | 585 | 6302 | 189 | 1771 | 71 | 2439 | 17,828 | 845 |
| EDB W-85 | 4.0 | 9794 | 784 | 5902 | 177 | 4385 | 175 | 2026 | 22,107 | 1136 |
| L.S.D. .05 | | 4162 | | 3755 | | 1739 | | 374 | 2608 | |
| L.S.D. .01 | | 6902 | | 6227 | | 2882 | | 620 | 4326 | |

[a]Based on a price of $.08/pound for U. S. No. 1, $.03 for U. S. No. 2, and $.04 for jumboes.

Table 3. Yield and market value of sweetpotatoes in various grades. Cacciatori test, Mira Loma, California.

| Treatment | Dosage (gallons/acre) | U. S. No. 1 yield | U. S. No. 1 value | U. S. No. 2 yield | U. S. No. 2 value | Jumboes yield | Jumboes value | Culls yield | Total yield | Total value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Yield in pounds/acre and value in dollars[a] | | | | | | | | |
| None | --- | 7336 | 587 | 2422 | 73 | 7004 | 280 | 5715 | 22,477 | 940 |
| DBCP | 0.75 | 9949 | 796 | 4321 | 130 | 7336 | 293 | 5419 | 27,025 | 1219 |
| DBCP | 1.0 | 7893 | 631 | 3955 | 119 | 7562 | 302 | 5349 | 24,760 | 1052 |
| EDB W-85 | 4.0 | 8346 | 668 | 3241 | 97 | 6377 | 255 | 4443 | 22,059 | 1020 |
| Telone | 15.0 | 10,803 | 864 | 4164 | 125 | 6621 | 265 | 4652 | 26,241 | 1254 |
| L.S.D. .05 | | 2273 | | 1014 | | n.s.[b] | | n.s. | n.s. | |
| L.S.D. .01 | | 3186 | | 1422 | | | | | | |

[a]See Table 2 for market price of each grade.
[b]No significant differences between treatments.

No. 2's and cull sweetpotatoes (Table 2).

Total yield of roots was significantly less on soil treated with 1.5 gallons/acre of DBCP; however, the percentage of No. 1 roots was increased from 16 on the nontreated plots to 41 on those treated with DBCP. The increase in yield of U. S. No. 1 roots on soil treated with DBCP, although 3630 pounds/acre, was not statistically significant at the .05 level.

The increase of 6106 pounds/acre of No. 1 roots on soil treated with EDB was significantly different from the nontreated at the .05 level. The highly significant increase in U. S. No. 2 roots in untreated soil is a reflection of damage by root-knot nematodes resulting in reduced size and quality of roots.

In the Cacciatori test, fumigants were applied to blocks 12 feet x 72 feet replicated five times in a latin square design. Yield of roots was obtained from two rows 50 feet long in each plot. As in the Ellis test, the roots were graded by the grower and the weights of roots in each grade are presented in Table 3. Soil samples were not obtained for nematode counts. Roots of plants in the check plot examined for evidence of root-knot nematode at the time of harvest showed a moderate level of infestation.

In this test the reduced dosages of DBCP (0.75 and 1.0 gallons/acre) did not cause a reduction in total yield. The highest total yield obtained was on soil treated with a 0.75 gallon/acre of DBCP. However, differences in total yields between the various treatments were not statistically significant at the .05 level. The increase in yield of U. S. No. 1 roots on soil treated with 0.75 gallon/acre of DBCP was significant at the .05 level, and with 15 gallons/acre of 1,3-dichloropropene (Telone) was significant at the .01 level. The increased yield of U. S. No. 2 roots on treated soil in this test is largely due to small size rather than to nematode injury. There was no significant difference in the weight of cull roots obtained from treated and untreated soil. The moderate nematode infection did not cause serious surface cracking of the roots.

In the Scarroni test the fumigants were applied to blocks 12 feet x 160 feet replicated five times in a randomized block design. Yields were obtained from one row 25 feet long in each plot. Total yield was the only data obtained, and no effort was made to separate the roots into the various grades. No root-knot nematode injury was observed on the roots. The results of this test are shown in Table 4. Total yields of roots in DBCP and EDB treated soil were somewhat higher than those on untreated soil, but these differences were not statistically significant.

Table 4. Total yield of sweetpotatoes in pounds/acre. Scarroni plot, Chino, California.

| Treatment | Dosage (gallons/acre) | Yield (pounds/acre) |
|---|---|---|
| None | -- | 15,752 |
| DBCP | 0.75 | 17,913 |
| DBCP | 1.25 | 18,331 |
| EDB W-85 | 3.5 | 19,586 |
| | | n.s.[a] |

[a]No significant differences between treatments.

## DISCUSSION

DBCP at 1.5 gallons/acre reduced the total yield of roots in the Ellis test. Dosages below this level did not have an adverse effect on total yield in the other two tests. In the Ellis test, where root-knot nematodes seriously affected yield and quality of roots, the yield of U. S. No. 1 roots was larger on the DBCP-treated soil than on the untreated soil and resulted in an increased total value of the crop. However, this increase was not nearly so great as that obtained on soil fumigated with EDB where an investment of approximately $25 in fumigant returned $346.

Telone appeared to be a satisfactory preplant soil fumigant for sweetpotatoes. In the one test where it was included there was a highly significant increase in the yield of U. S. No. 1 roots. Although the test area had only a moderate root-knot nematode infestation, approximately $25 worth of fumigant returned $314.

Two factors which may be of importance in the yields obtained on DBCP-treated soil were the soil types treated and the waiting period between treating and planting. All soils were sandy loam soils low in organic matter and relatively warm (62° to 75° F) at the time of treatment. The period between treating and planting was 3 to 4 weeks in all tests.

The results of these tests indicate that DBCP warrants further testing as a fumigant for preplant treatment for soil to be planted to sweetpotatoes. The tests need to be established under a wider range of conditions than occurred in the present investigations.

One other item of interest that is pointed up by these tests is that the phytotoxicity of a soil fumigant to a particular crop cannot always be accurately determined if plots are established only on soils where nematodes are a serious problem. The response to nematode control, as represented by the increased weight of U. S. No. 1 roots in the DBCP-treated soil in the Ellis test, may mask the adverse effects of the fumigant on the crop.

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# FIRST YEAR EFFECTS OF 10 SELECTED CYTOSPORA ISOLATES ON 20 FRUIT AND FOREST TREE SPECIES AND VARIETIES[1]

A. W. Helton[2]

## Summary

Ten Cytospora isolates from fruit and forest trees were inoculated to 8 forest or ornamental species and 12 cultivated or native fruit types. Nine of the isolates were pathogenic on one or more hosts, and three isolates invaded golden willow with spectacular results.

The investigation demonstrated that Cytospora fungi are nonspecific organisms capable of infecting a wide range of host plants. The data suggest, however, that inoculum from forest and ornamental trees is much less a threat to orchard trees than inoculum from infested orchards.

## INTRODUCTION

In a preliminary investigation of the pathogenicity of selected Cytospora isolates from stone fruits, the fungi demonstrated various degrees of virulence when inoculated into healthy plum and peach trees (6). The results were in agreement with those of some stone fruit investigations reported in the literature but not with others. This inconsistency necessitated an evaluation of reports dealing with other hosts.

Apparently similar, if not identical, isolates have been reported to be pathogenic on pome fruit trees (8) as well as stone fruits. Others have been known to occur on hazelnut (16, 23) and Japanese chestnut (18) trees. A number of forest and ornamental species also have been damaged by Cytospora fungi (1, 2, 5, 7). Primary parasitism, in connection with some predisposing or contributing factor, has been noted for poplars (15, 19, 20), Chinese and other elms (3, 13), weeping and other willows (9, 21), sycamore (14), sugar and other maples (12, 22), cottonwood (27), larch (10), Italian cypress (28), cedar (17), Rocky Mountain Douglas fir and true firs (25, 26), spruce (11), pines (4), and other conifers (24).

The preliminary investigation in Idaho revealed that isolates from plum and cherry trees were vigorously pathogenic on other stone fruit varieties as well as on the varieties from which they were isolated. Since reports in the literature indicated that many other kinds of trees were affected by Cytospora fungi, questions arose regarding the effects of isolates from orchard trees on other suscepts commonly found near orchards in Idaho, and of inoculum from such hosts on cultivated orchard trees.

## MATERIALS AND METHODS

Ten isolates were selected from among the Cytospora fungi placed in the Idaho culture bank between 1955 and 1959. These represented a range of host trees of both fruit (cultivated and noncultivated) and forest types (Table 1). The isolates were maintained on malt agar medium at 25° ± 1°C for 2 months, ending with their inoculation to the test trees on June 16, 1959.

The test trees had been planted on a 6 x 12-foot grid in a field plot in April 1957. When the trees were inoculated they were 3 to 4 years old from the seed, including the grafted orchard varieties. Height, trunk diameter, and other growth factors differed according to species and/or variety habit, but all were in a vigorous condition at the time of inoculation.

The species and varieties selected to represent the cultivated or semi-cultivated (for example, used as orchard tree rootstocks and/or producing similar fruits) fruit tree group were Italian and Stanley prune and President plum (*Prunus domestica*), Myrobalan plum (*P. cerasifera*), Montmorency sour cherry (*P. cerasus*), Bing sweet cherry (*P. avium*), chokecherry (*P. virginiana*), Mahaleb cherry (*P. mahaleb*), Chinese apricot (*P. armeniaca*), Lovell peach (*P. persica*), Bartlett pear (*Pyrus communis*), and Red Rome Beauty apple (*Malus pumila*). Those representing the forest and ornamental group were Russian olive (*Elaeagnus angustifolia*), golden willow (*Salix alba*), black hawthorn (*Crataegus douglasii*), Sitka Mountain ash (*Sorbus sitchensis*), Douglas maple (*Acer glabrum* var. *douglasii*), European white birch (*Betula alba*),

[1]Approved by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 513.
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Table 1. Sources of isolates.

| Isolate | Date isolated | Host type | Host location |
|---|---|---|---|
| Cy-3 | Oct. 1955 | President plum | Orchard; Marsing vic. |
| Cy-14 | Oct. 1957 | poplar | Backyard; Moscow |
| Cy-16 | Feb. 1955 | Italian prune | Orchard; Fruitland vic. |
| Cy-20 | Mar. 1957 | willow | Forest area; Tamarack vic. |
| Cy-29 | Apr. 1957 | Russian olive | Farm windbreak; Moscow vic. |
| Cy-32 | July 1957 | Jonathan apple | Orchard; Parma vic. |
| Cy-34 | July 1957 | Hale peach | Orchard; Marsing vic. |
| Cy-36 | July 1957 | Bing cherry | Orchard; Emmett vic. |
| Cy-40 | June 1959 | apple | Orchard; Washington State |
| Cy-42 | June 1959 | Italian prune | Experiment plot; Moscow vic. |

Table 2. Pathogenicity of isolates on the host species and/or variety (or closely related variety) from which each was isolated, and hosts most extensively invaded by each isolate.

| Isolate | Same or closely related host[a] | Most extensively invaded host: host | Most extensively invaded host: canker area[a] |
|---|---|---|---|
| Cy-3 | 54 | golden willow | 347 |
| Cy-14 | 0 | golden willow | 462 |
| Cy-16 | 13 | President plum | 34 |
| Cy-20 | 0 | all inoculated | 0 |
| Cy-29 | 84 | Russian olive | 91 |
| Cy-32 | 0 | Stanley prune | 32 |
| Cy-34 | 24 | golden willow | 462 |
| Cy-36 | 25 | Bing & Montmorency cherry | 34 |
| Cy-40 | 0 | President plum | 38 |
| Cy-42 | 0 | Lovell peach | 19 |

[a]Canker area in $cm^2$; average of the two most severely infected of six replicates.

Norway spruce (Picea abies), and a hybrid poplar (Populus sp.).

Inoculations were made by inserting approximately 0.5 $cm^2$ of agar, covered with fungus growth, under bark flaps or by pressing the agar into cuts through the bark and outer xylem. Each tree-isolate combination was replicated six times (Table 2). All inoculation wounds were bound with elastic tape. Control trees received sterile agar.

Degree of infection was determined by canker-area measurements at infected inoculation sites with a centimeter tape. Gummosis at infected sites was common but not considered when canker areas were determined. Pycnidial pustules were common in canker surfaces, but not always present, and they did not affect recorded measurements. All measurements were made on August 8, 1959.

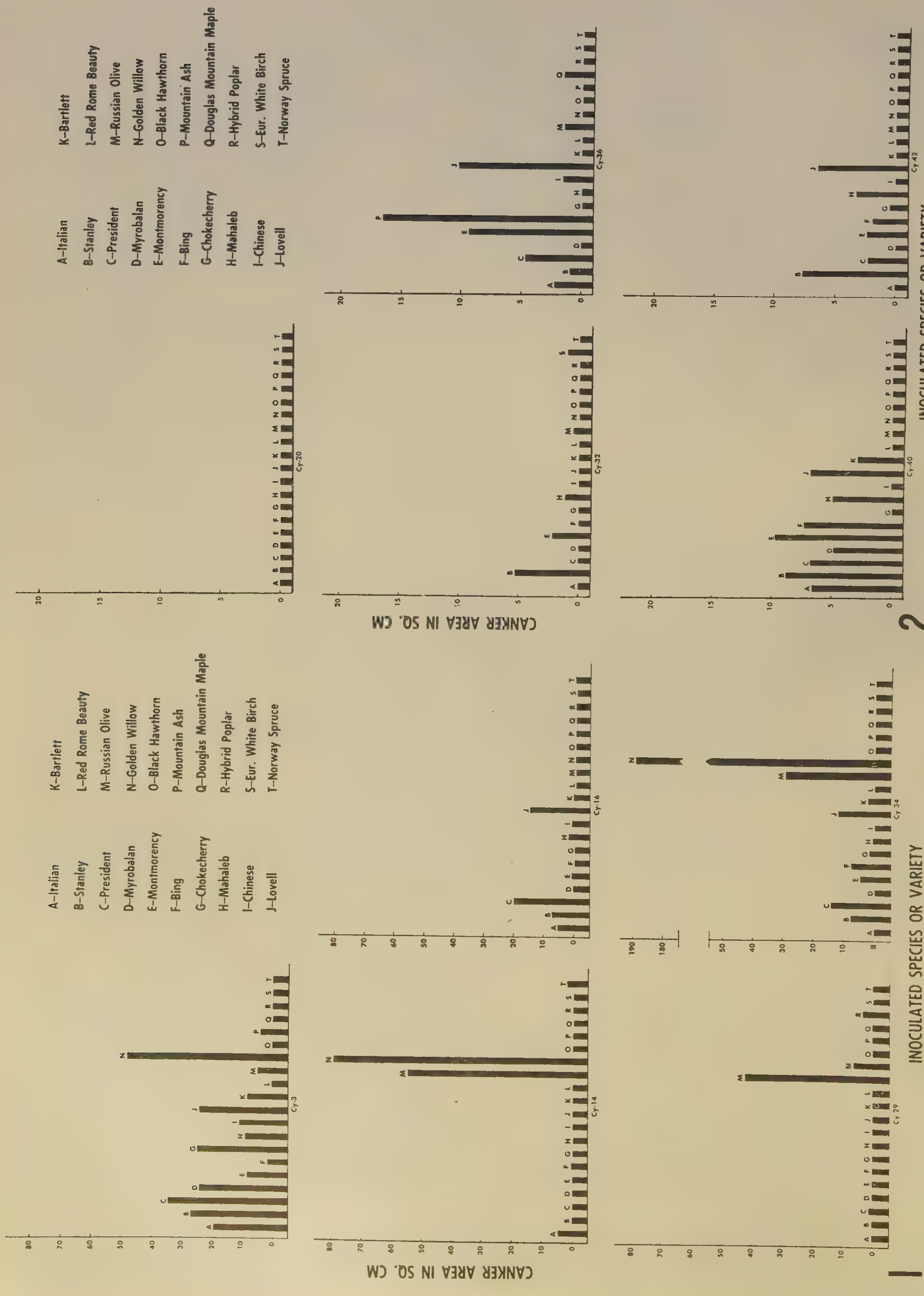
## RESULTS

No infections developed on the control trees.

Pathogenicity of the 10 isolates varied from strictly saprophytic behavior to strong virulence. Five isolates failed to induce disease on trees of the same variety (or closely related variety) from which they were isolated (Table 2), but only one (Cy-20) failed to infect any of the hosts to which it was inoculated.

Only 2 of the 10 isolates produced their most virulent infections on the varieties from which they were isolated. These were Cy-29 from Russian olive and Cy-36 from Bing cherry (Tables 1 and 2). Golden willow was spectacularly injured by three of the isolates (Table 2). Russian olive was almost as spectacularly injured by two of the same isolates and one other. President plum sustained prominent injury by two isolates, but the degree of pathogenesis was much less than that on golden willow.

Isolates from cultivated and semi-cultivated fruit trees caused most damaging infection on inoculated trees of the fruit tree group. These isolates were Cy-3, Cy-16, Cy-32, Cy-34, Cy-36, Cy-40 and Cy-42 (Figs. 1 and 2). One (Cy-34) was prominently pathogenic also on Russian

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olive and golden willow. Two of the isolates taken from forest species (Cy-14 and Cy-29) were most frequently infectious, and produced most serious disease, on trees of the forest tree group.

Isolates Cy-16, Cy-40 and Cy-42 failed to infect trees of the forest group except for slight invasion of Norway spruce by Cy-16 (Fig. 1). Cy-34 infected only two forest types, namely Russian olive and golden willow. This isolate was more spectacular on golden willow than any other isolate on any host.

Cy-29 (from Russian olive) infected none of the fruit trees except President plum (Fig. 1).

Red Rome Beauty apple and black hawthorn trees were not invaded by any of the 10 isolates (Figs. 1 and 2).

## DISCUSSION AND CONCLUSIONS

Not all isolates infected golden willow, but those that did produced spectacular results. Canker expansion proceeded at such a rate that blackened stems were discernible from several rows away within a few days. Flagging was accomplished promptly also, and the stems often broke at inoculation sites. Since cuttings root readily and grow rapidly, golden willow may prove valuable as a test plant for screening potential control chemicals.

The results suggest that most Cytospora fungi can be expected to infect trees of a number of species and varieties, both fruit and forest-ornamental. However, the data show that isolates from one group generally are not very damaging to trees of the other group. The evidence indicates, therefore, that backyard, windbreak, and forest trees probably are not the threat to fruit orchards that they were suspected of being. Nevertheless, insufficient information has been accumulated to reveal whether infection of trees of one group by isolates from trees of the other group can lead to later development of serious Cytospora problems in the second group by adaptation of fungus strains or mutations of the kind known to occur commonly in the laboratory. These possibilities indicate that beneficial exclusion measures (for example, surgery in the orchard) should continue to receive emphasis as preventive measures.

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FIGURE 1. Effects of isolates Cy-3, Cy-14, Cy-16, Cy-29 and Cy-34 on 12 fruit tree species or varieties and 8 forest species. Each bar value is the average of six replicates.

FIGURE 2. Effects of isolates Cy-20, Cy-32, Cy-36, Cy-40 and Cy-42 on 12 fruit tree species or varieties and 8 forest species. Each bar value is the average of six replicates.

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# CITRUS NEMATODE ON AMERICAN PERSIMMON IN ISRAEL[1]

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The citrus nematode, Tylenchulus semipenetrans Cobb, 1913, was first found infesting citrus roots in Israel as early as 1913 by Aaron Aaronsohn, who despatched samples to Washington for identification by Cobb (1). It has since been recognized as an important parasite on roots of the three main citrus rootstocks in Israel, sour orange (Citrus aurantium), sweet lime (C. aurantifolia var. dulcis) and rough lemon (C. limon).

Arrested growth and severe chlorosis were observed during the summer of 1960, affecting four persimmon varieties (Hachiya, Triumph, Tamopan, and Fuyu) on native American persimmon rootstock (Diospyros virginiana), the common rootstock for all persimmon varieties in Israel. The trees were part of a mixed orchard of several tropical and sub-tropical fruit tree varieties, established on the site of an uprooted orange grove in Acre, northern Israel. In September 1960, at the end of their second year in the orchard, most of the persimmon trees were undersized and their leaves dropped prematurely.

Root and soil samples were taken twice from all the fruit tree varieties in the orchard, in the autumn and winter of 1960-61.

Larvae, males and attached females with egg masses of T. semipenetrans were found parasitizing the roots of the persimmon rootstock in 18 of 20 root samples. Larvae were recovered from all 20 soil samples. No additional parasitic nematode species were identified from soil samples and none were recovered from the persimmon roots processed by Young's incubation method (5).

Nesbitt (3) and Raski, et al. (4) have reported citrus nematode infesting roots of the Chinese persimmon, D. lotus. At the time of these findings there appears to be no record of D. virginiana serving as host to T. semipenetrans.

No larvae or females of T. semipenetrans were recovered from the roots of the following fruit tree species in the same orchard: anona (Annona hybrida), avocado (Persea gratissima), banana (Musa cavendishii), guava (Psidium guajava), loquat (Eriobotrya japonica), and mango (Mangifera indica). Direct examination of the roots of the following weeds were also negative for the citrus nematode: elephant grass (Pennisetum purpureum), dwarf nettle (Urtica urens), mallow (Malva sp.) and nut grass (Cyperus rotundus).

All the banana root samples, processed by Young's incubation method, were heavily infested with spiral nematodes, Helicotylenchus multicinctus (Cobb, 1893) Golden, 1956; these nematodes are universal parasites in all banana orchards in Israel (2). The same nematode species were also present in soil from the rhizosphere of the sampled avocado trees.

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# THE IMPORTANCE OF ROOT GRAFTS IN OAK WILT SPREAD IN MISSOURI

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Local spread of the oak wilt fungus (Ceratocystis fagacearum (Bretz) Hunt) causes many of the new infections in oaks. About 60% of the wilting trees in Missouri each year are within 50 feet of previously infected trees[3]. It is widely accepted that most local disease spread is the result of movement of fungus spores from diseased trees to adjacent healthy trees through root grafts. Many control recommendations emphasize measures designed to prevent this mode of fungus transmission. However, recent investigations of the pattern and frequency of root grafting between oaks in Missouri suggest that such underground connections occur too infrequently to account for much of the short-distance oak wilt spread that does occur.

These studies were conducted in poletimber and sawtimber oak-hickory stands at five locations in southeastern Missouri. A variety of stand densities, sites, slopes, and aspects is represented within the areas. Oak wilt infection and local spread of the disease are prevalent at all five locations.

Healthy black oaks (Quercus velutina) and white oaks (Q. alba), 4 inches d.b.h. and larger, were selected within these areas so that the minimum distance between sample trees was two chains and the minimum distance between a tree and a road or edge of the stand was three chains. The selected trees were cut during the growing season. A tracer solution or tracer solution plus endoconidia of a white mutant strain of the oak wilt fungus were immediately introduced into the stumps by means of the technique described by True, et al.[4]. In brief, this technique consists of fashioning a watertight, tar-paper collar around the top of the stump to provide a reservoir for the solution until it is taken up by the stump.

In the first study, 25 black oaks and 25 white oaks were treated during late July and early August 1957. The tracer solution used in these trees was 1 pint of saturated copper sulfate in 1 gallon of water per 12 inches of stump diameter. Symptoms of copper injury in the foliage of nearby oaks were evidence of root connections between the affected and treated tree. As soon as copper injury was observed in the foliage of a tree, that tree was cut and the stump treated with copper solution in the manner previously described.

Few root grafts were found in this study. Only 16% of the black oaks and 20% of the white oaks were root-connected to another tree. Each graft was between trees of the same species. In only one instance were more than two trees involved in a graft series. In this case four black oaks were involved. When the first of this group was treated, one adjoining tree showed injury. When this second tree was treated, two additional trees showed injury. Treatment of these third and fourth trees produced no evidence of further root grafting. The longest distance between grafted trees or the most widely separated trees in a series of grafted trees was 36 feet. This occurred between two white oaks. The average distance between grafted trees was 11 feet.

The disadvantage in using copper sulfate to trace root grafts is that the trees involved are killed or severely injured and thus there is no opportunity to determine how many of the root grafts revealed may be functional for spread of the fungus. Therefore, in the second study the tracer material used was a 0.5% solution of azosulfamide, a red organic dye, applied at the rate of 1 quart per inch of stump diameter. A concentrated suspension of endoconidia of a white mutant strain of the oak wilt fungus was added to this solution. (Preliminary studies proved azosulfamide solutions to be readily translocated in the vascular system of oaks and to be non-toxic to oaks or the oak wilt fungus in concentrations of up to 2%.) Eighty-nine black oaks and 86 white oaks were treated in four areas during June, July, and August 1958. Coloration in the foliage and vascular tissues of nearby oaks was evidence of the presence of root grafts. Subsequent development of disease symptoms in and isolation of the fungus from such trees was evidence of root grafts functional for disease spread.

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4 True, R. P., T. M. Judy, and Eldon Ross. 1955. The absorption of solutions through the tops of freshly cut oak stumps. West Virginia Agr. Exp. Sta. Current Rept. No. 11, 7 pp.

The incidence of root grafting revealed in this study was similar to that in the first investigation. On the basis of translocation of dye, 13% of the black oaks and 16% of the white oaks were root-grafted to other trees. Each graft was between trees of the same species, with one exception. In this instance, a black oak was grafted to a blackjack oak (Q. marilandica). All grafts involved only the treated and one additional tree, with two exceptions. In one case, a white oak was grafted to two other white oaks; and in the other case, a white oak was grafted to three other white oaks. The maximum distance between grafted trees was 25 feet and the average distance was 8 feet.

In the 2 years since these trees were treated, only 3 of the 29 trees known to be connected to treated trees on the basis of dye translocation have developed disease symptoms. Two of these are black oaks and one is a white oak. The white strain of the oak wilt fungus was recovered from each of them. Attempts to isolate the fungus from the other trees known to be graft-connected to treated trees have not been successful. Thirteen other oaks within 50 feet of treated trees, but in which no dye symptoms have been observed, displayed wilt symptoms in the second year after treatment. The white strain of the fungus could be isolated from only two of these. The possibility is remote that root grafts were involved in the spread of the fungus into these two trees since in one case it would require a graft between a black and scarlet oak (Q. coccinea), and in the other case between a black and white oak.

There is no measure of the effectiveness of copper sulfate or azosulfamide solutions for detecting all existing root grafts between treated and other trees. However, in the absence of contrary evidence, the tentative conclusion is that the incidence of root grafting in oaks in the Missouri Ozarks is approximately 17%. Grafts between oaks of the red group and oaks of the white group were not detected and must occur only rarely. Grafts between oaks of different species but within a species group appear to be very infrequent. Graft series involving more than a pair of trees are not common. Moreover, only about 2% of the inoculated oaks were connected to other oaks by root grafts functional for disease spread. Thus, the incidence of root grafting indicated by these studies is far too low and the pattern of grafting far too limited to account for much of the short-distance oak wilt spread that does occur in Missouri. Other factors or agents responsible for local spread are indicated.

CENTRAL STATES FOREST EXPERIMENT STATION, FOREST SERVICE,
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# INJURY TO NARCISSUS FROM TREATMENT OF BULBS WITH CERTAIN MERCURY COMPOUNDS[1]

C. J. Gould, W. D. McClellan, and V. L. Miller[2]


## Summary

The types of injury to narcissus plants following bulb dips in certain ethylmercury compounds are described. The most common injury is a distortion of flowers, but stunting and distortion of leaves may also occur and occasionally a black internal disintegration of the bulbs. Delayed treatment of bulbs and rapid drying after treatment have decreased the injury. However, because of its lack of phytotoxicity at fungicidal rates, as well as its lower cost, phenylmercury acetate has generally replaced ethylmercury compounds for treatment of narcissus bulbs for the control of Fusarium oxysporum Schlecht. f. narcissi Snyd. & Hans. in the United States.


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Soon after digging, commercial growers usually dip narcissus bulbs in a solution or a suspension of an organic mercury compound for the control of basal rot (Fusarium oxysporum Schlecht. f. narcissi Snyd. & Hans.) (3). At this time the flower and foliage primordia for the following year are already present in the center of the bulb. Under certain conditions the use of some mercury compounds has resulted in a distinctive type of injury which has been mentioned or briefly described by various workers (1, 3, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16). This article describes more completely the symptoms of such mercury injury to narcissus briefly reported by the authors (4) in 1949.

The symptoms were observed in commercial practice as well as in experimental work while testing different mercury compounds over a period of several years. The most commonly tested mercurials and range of rates are listed in Table 1. Bulbs were dipped at various

Table 1. Most frequently tested mercurials and amounts used.

| Active ingredient | Range of concentrations tested in ppm of Hg | Rate often recommended per 100 gallons | Rate often recommended as ppm |
|---|---|---|---|
| Ethylmercury chloride (2% Ceresan) | 151-275 | 12.5 lb | 229 |
| Ethylmercury phosphate (New Improved Ceresan) | 103-450 | 2.5 lb | 109 |
| N-(ethylmercuri)-p-toluene sulfonanilide (Ceresan M) | 64-326 | -- | -- |
| Phenylmercuri triethanol ammonium lactate (Puratized Agricultural Spray) | 77-667 | 1.0 gal. | 200 |
| Phenylmercury acetate (Mersolite 8) | 89-458 | 0.2 lb | 144 |

times after digging, at different temperatures, with and without wetting agents, for various lengths of time, and so forth, depending on the experiment. The variety generally used was King Alfred and the types of injury described pertain to that variety. Flowers of The First and Sir Watkin varieties also showed similar symptoms following treatments with ethylmercury chloride and ethylmercury phosphate.

## TYPES OF INJURY

Bulbs: Death, followed by a black disintegration of the flower primordia and adjacent bulb scales, was observed in both commercial and experimental lots of bulbs harvested the summer following treatment of bulbs in the fall of the preceding year (Fig. 1). The severely affected centers of bulbs turned into black jelly-like masses which later dried, leaving the bulbs with hollow black-walled interiors. A very severe case of this type was observed the year after a

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FIGURE 1. Treated King Alfred narcissus bulbs showing varying degrees of ethylmercury injury.

FIGURE 2. Injured flowers from narcissus bulbs treated with ethylmercury. Normal flower at left.

commercial stock of narcissus bulbs was treated with ethylmercury chloride and kept moist in closed storage without air circulation for about 2 weeks before planting. The same type of injury, produced experimentally on Washington-grown bulbs treated with ethylmercury phosphate 3 days after digging in 1947, was evident when the bulbs were harvested in 1948. Injury also occurred in experiments with Long Island-grown bulbs, and was evident following storage. However, bulb injury has been observed much less frequently than flower injury.

Leaves: Leaves from treated bulbs were occasionally yellow-green, somewhat speckled, slightly roughened or swollen near the tips, and occasionally stunted.

Flowers: Flower distortion was the most common indication of mercury injury to narcissus bulbs (Fig. 2). The following changes were noted: perianth segments were paler than normal and were proportionately narrower and smaller; the trumpet was paler and smaller, often lacked the typical recurved edge, and was frequently constricted around the excerted stigma and anthers; blasted flowers represented the extreme degree of flower injury. The average blooming date was sometimes accelerated by mercury treatments of bulbs in the tests reported here and in tests reported by Specht, et al. (13). In contrast, Hawker (6) obtained a retardation in flowering following treatment of bulbs with mercury chloride at 770 ppm of mercury.

## RELATION OF TREATMENT TO INJURY

Time of Treatment: The phytotoxic effect of mercury compounds was much greater on Washington-grown bulbs treated within 3 days after digging than on those treated later. For

instance, bulbs treated in 1946 with N-(ethylmercuri)-p-toluene sulfonanilide 3 days after digging, and later forced in a greenhouse, produced flowers in which the trumpet diameter averaged 33 mm. Similar bulbs treated 10 days after digging produced flowers whose trumpet diameter averaged 40 mm. The trumpet diameter of flowers from untreated bulbs averaged 44 mm. The reduced injury associated with a delay in bulb treatment has been noted also by Weiss, et al. (15) for flowers and by Aycock (1) for bulbs. Unfortunately, such delayed treatment has usually been accompanied by increased losses from basal rot, particularly in warm climates.

Drying Bulbs Rapidly after Treatment: Injury was reduced by rapidly drying the bulbs soon after they were treated. For example, in the experiment mentioned in the preceding paragraph, the average trumpet diameter was 42 mm when the bulbs were treated 3 days after digging and dried rapidly for 3 days with 95° F heat under forced ventilation, as compared with 33 mm when similarly-treated bulbs were not dried rapidly. The average trumpet diameter in lots not treated until 10 days after digging was 45 mm when drying was rapid and 40 mm when it was slow. Such drying probably decreased the amount of penetration of the mercury compound. This principle was first observed and put into commercial practice on Long Island, New York, by Dr. Frank A. Haasis.

Type of Compound: When used at concentrations strong enough to control basal rot, the organic mercury compounds tested varied in their phytotoxic effect. Ethylmercury chloride, ethylmercury phosphate, and N-(ethylmercuri)-p-toluene sulfonanilide sometimes caused severe injury, whereas injury was lacking when either phenylmercury acetate or phenylmercuri triethanol ammonium lactate was used. Phenylmercury acetate has been used with mercury concentration as high as 458 ppm without any appreciable phytotoxic effects. The fungicidal and the phytotoxic action of organic mercury compounds are influenced by the organic radical. Thus, in the experiments, there was a similarity in the injurious action of the ethylmercury compounds, which may be attributed to the action of the common ethylmercuric ion. Similarly, the phenylmercury compounds dissociate giving the phenylmercuric ion. Both ethyl- and phenyl-mercuric radicals are active fungicidally but the ethylmercury radical causes phytotoxicity. An interesting sidelight is the fact that ethylmercury phosphate was frequently more phytotoxic than ethylmercury chloride, even though the mercury content of the latter was usually 229 ppm as compared with only 109 or 114 ppm in the former. This may be due to a difference in speed of solubility. Aycock (1) obtained considerable mercury injury to bulbs and flowers from use of N-ethylmercuri-1,2,3,6-tetrahydro-3,6-endomethano-3,4,5,6,7,7-hexachlorophthalamide (Emmi) at 180 ppm mercury, but only an occasional slight bulb discoloration and no flower injury with phenylmercury acetate.

## DISCUSSION

Mercury can be absorbed directly by narcissus, as shown both by spectro-chemical and chemical analyses. Thus, spectro-chemical determinations by Specht and co-workers (9, 12, 13) of mercury in plants grown from narcissus bulbs dipped in mercurial solutions or suspensions showed the highest concentrations of mercury in the center and basal plate of the bulb and least in the leaves. The degree of injury was generally correlated with the amount of mercury absorbed (9, 12, 13). Chemical tests by Miller, et al. (11) showed that the bulbs immediately after being dipped contained most of the mercury in the husk and basal plate. Specht, et al. (13) found that the upper part of the bulb was the part most "disturbed" in composition of Fe, Mn, Cu, Al, P and Ca by mercurial treatments. These workers were not certain, however, that a disturbed nutrient-element balance was necessarily the cause of flower injury.

Aycock (1) has pointed out that varieties differ in susceptibility to injury. Thus, in his tests, the First variety was much more susceptible than were King Alfred and Flower Carpet varieties.

When used at the recommended fungicidal concentrations, phenylmercury compounds were never phytotoxic in the tests reported here, nor were formulated mixtures containing as much as 1121 ppm mercury as phenylmercury acetate (11). However, ethylmercury compounds often caused considerable injury, especially when used on bulbs that were immature or when used on bulbs too soon after digging. Booer (2) found phenylmercury compounds to be less toxic than ethylmercury compounds for soil treatment.

Because of the lower cost of phenylmercury acetate and its lack of phytotoxicity at effective rates, this compound has been adopted widely by commercial narcissus growers in place of the ethylmercury compounds. It is usually applied (3, 8) as a dip at a rate of 1 pound in 500 to

800 gallons (143-89 ppm mercury) of water for 5 to 15 minutes. A double treatment (within 10 days after digging and again just before planting) is recommended for severely infected stocks, and a single treatment (after the bulbs are cleaned) for slightly infected stocks in the Pacific Northwest. Even the double treatment with phenylmercury acetate has produced no apparent injury, nor has there been any noticeable cumulative effect over a 3-year period of consecutive treatments.

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# SOME HOST-PARASITE RELATIONSHIPS IN THE CURVULARIA DISEASE OF GLADIOLUS IN FLORIDA[1]

Curtis R. Jackson

Abstract

Some soil relationships in the gladiolus disease caused by Curvularia trifolii were investigated by incubating potted plants in infested soil at various constant temperatures. Favorable temperatures for growth of the pathogen in vitro and for foliage infection were also determined. Mycelial weight in stationary cultures was significantly greatest at 28°C. Foliage infection of three varieties occurred at 24° to 36°. Soil infestation by means of infected plant debris, infected corms, and conidia was demonstrated and the results indicated that the fungus may persist in the soil in the absence of host crop debris. Roots were frequently infected over a range of 12° to 36° while corm infection did not occur below 16°. A diagrammatic outline of the pertinent cycles that probably occur in Florida is given.

## INTRODUCTION

The Curvularia disease of gladiolus causes substantial losses in Florida by reducing the yield and quality of corms and flowers and by increasing production costs. Commercial control of the leaf and flower spotting phase of the disease has been achieved through use of protective fungicides. The present study was initiated in 1958 to clarify certain aspects of the life cycle of the pathogen. This report presents results of investigations of the relationship of temperature to foliage, corm, and root infection and of the establishment of the fungus in the soil.

## LITERATURE REVIEW

In 1948 Magie (2) reported a new leaf spot disease of gladiolus caused by Curvularia and he later reported (3) that corms were attacked by the fungus. By 1956, according to Van Weerdt (8), the disease had been reported from 17 States and several foreign countries. Magie (3) and others referred to the gladiolus pathogen as Curvularia lunata (Wakk.) Boed. Luttrell (1) in 1956 suggested that the form of conidial hilum served to distinguish between C. lunata and Curvularia trifolii (Kauff.) Boed. and he found gladiolus isolates to be C. trifolii. Parmelee (7) found that isolates of C. trifolii from gladiolus and clover were pathogenically distinct and proposed the name C. trifolii f. sp. gladioli Parmelee & Luttrell for the form pathogenic on gladiolus.

McClellan and Marshall (4) reported that Curvularia from gladiolus grew best in culture from 75° to 85°F, with a temperature range for foliage infection (Picardy variety) of 45° to 85° and an optimum of 75° to 85°. Van Weerdt (8) reported that optimum growth in pure culture occurred at 28°C. The relative susceptibility of gladiolus varieties to foliage infection was discussed by Magie (3) and McClellan and Pryor (5). Magie (3) also pointed out that mature foliage of plants from seeds and cormels was more susceptible than mature foliage from large corms.

Magie (3) and Mendiola-Ela (6) recognized that the fungus persisted in the soil and in infected plants left in the field. Mendiola-Ela (6) suggested that the fungus becomes incorporated in the soil when infected corms rot and that it also remains as spores and thick-walled hyphae in infected crop debris in the soil. Van Weerdt (8) recovered the fungus from buried crop debris after 2 months but not after 9 months and was unable to detect spores in debris from which the fungus was recovered.

## RELATIONSHIP OF TEMPERATURE TO GROWTH IN VITRO

An aqueous medium composed of 180 ml of filtered V-8 juice, 1 g ammonium nitrate, and 1 g dibasic potassium phosphate/liter was used. One hundred-ml portions, in 500-ml Erlenmeyer flasks, were sterilized and seeded with 3-mm discs of a non-sporulating pure culture of C. trifolii from a gladiolus corm. Six flasks were assigned randomly to each of five chambers where constant temperatures of 16°, 20°, 24°, 28°, and 32°C were maintained. After 7 days stationary cultures were harvested. The mycelial contents of each flask were collected on dry, previously weighed filter papers and dried at 32°.

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The dry weight of mycelia which developed at 28° C was significantly greater than dry weights at other temperatures. Growth decreased in the order 24°, 32°, 20°, and 16° with no significant difference between 24° and 32°.

## RELATIONSHIP OF TEMPERATURE TO FOLIAGE INFECTION

Methods: The foliage of the gladiolus varieties Corona, Valeria, and Florida Pink was used to determine the most favorable temperature range for infection. Eight-week-old field-grown plants of Corona and Valeria from large corms (#1) were decapitated and the cut ends of the foliage were placed in water in small jars. Mature greenhouse grown plants of Florida Pink from small corms (#5) growing in individual 4-inch plastic pots were also used.

An aqueous suspension of conidia from pure cultures of three isolates (two corm, one leaf) from gladiolus was atomized on the foliage of all varieties. All plants and containers were sealed in separate polyethylene plastic bags with wet paper toweling to help maintain high humidity. Three inoculated plants and one uninoculated plant of each variety were placed in each of six lighted chambers at constant temperatures of 16°, 20°, 24°, 28°, 32°, and 36° C. After 4 days all plants were removed from the chambers and polyethylene bags and placed in a shaded part of the greenhouse at ambient temperatures of 16° to 28°. The numbers of developing lesions over 0.5 mm in diameter were counted 7 days after inoculation. Confluent lesions were not enumerated individually.

Results: Uninoculated foliage did not become infected with Curvularia and remained turgid throughout the experiment. A slight marginal yellowing of uninoculated Corona and Valeria foliage occurred during incubation at 36° C. Mean numbers of leaf lesions are given in Table 1. No infection occurred at 16° or 20°.

Table 1. Mean numbers of leaf lesions that developed after incubation at four constant temperatures.

| °C | Florida Pink | Corona | Valeria |
|---|---|---|---|
| 24 | 10 | 0 | 4 |
| 28 | 16 | 5 | 7 |
| 32 | 7 | 12 | 10 |
| 36 | 6 | 7 | 6 |

## CORM AND ROOT INFECTION

Curvularia infection of gladiolus corms is most commonly found in Florida during the fall and spring. Root infection is also prevalent but has not been shown to be related to any major crop failure. Results of preliminary experiments suggested that soil infestation occurred in several ways and showed that root or corm infection took place over a temperature range of 12° to 36° C. Sporulating colonies of the fungus found on artificially infested gladiolus spike tissue that had been buried in the field for 9 months indicated that crop debris could be a primary source of inoculum.

### Effects of Soil Infestation and Temperature on Infection of Gladiolus Corms and Roots

Methods: Twice-autoclaved sandy soil was used to fill 60 disinfested 4-inch plastic pots. The soil was infested with *C. trifolii* by: 1) pouring 50 ml of conidia suspension, containing about 300 conidia per ml, on the soil surface, and 2) burying artificially infested segments of gladiolus spike tissue vertically in the soil near the edge of each pot. The soil in 25 pots was infested in each of these ways and 10 uninfested pots served as controls.

Thirty days after the soil was infested, one #2 corm of Spic & Span variety was planted in each of the 60 pots. The corms were first husked (cataphylls removed), selected for freedom from blemishes, and disinfested in 0.26% sodium hypochlorite solution. When all sprouts had emerged, soil moisture was adjusted to approximately one-half of field capacity and groups of five replications of each infestation method and two controls were placed in large polyethylene bags, sealed, and put in one of five lighted chambers at a constant temperature of 16°, 20°, 24°, 28°, or 32° C.

Corm, root, and leaf symptoms were rated 23 days after the potted plants were placed in the chambers. Disease ratings were the sums of ratings of corms, roots and leaves. Root infection was rated from 0, no infection, to 4, all roots infected; corm ratings were based on the numbers and size of lesions. The numbers of leaf lesions, regardless of size, were added to the ratings. Each plant exhibiting symptoms was cultured on 2% potato-dextrose agar.

Results: Control plants, except those incubated at 28°C, did not become infected. At 28° control plants developed corm rot caused by Fusarium sp. and bacteria.

C. trifolii was recovered from 94% of the plants sampled. Fusarium spp. and bacteria were isolated occasionally. In such instances the disease ratings were adjusted to exclude symptoms clearly due to organisms other than C. trifolii. Symptoms and disease ratings are given in Table 2.

Table 2. Principal symptoms and mean disease ratings (five replications) of Curvularia infection of Spic & Span gladiolus at various temperatures.

| °C | Soil infestation methods: spore suspension | spike tissue |
|---|---|---|
| 16 | RB,[a] CL - 3.6 | RB, CL - 2.6 |
| 20 | RB, CL - 5.8 | RB, CL - 2.0 |
| 24 | RB, CR, LL - 10.8 | RB, CL LL - 10.8 |
| 28 | RR, CR, LL - 14.0 | RR, CR, LL - 9.6 |
| 32 | RR, CR, LL - 14.0 | RR, CR, LL - 13.0 |

[a]RB - root browning, CL - corm lesions, LL - leaf lesions, CR - corm rot, RR - root rot.

Analysis of disease ratings indicated significant differences at the 5% level among temperatures and between infestations methods. The interaction of these two factors was not significant. Soil infestation with the spore suspension resulted in greater disease production. Disease severity was greater at 24°, 28°, and 32°C than at 16° and 20°. No significant differences were found among disease ratings from plants incubated at 24°, 28°, and 32° or between 16° and 20° ratings.

Leaf lesions, which developed on many plants incubated above 20°C, were always associated with aerial sporulation on the necks (leaf bases at the soil level) of the infected plants. Abundant conidia also developed in and on decaying corms in the soil. Slight browning to complete rotting of root systems was due to Curvularia infection.

## Corm Source of Soil Infestation

Methods: Twenty-five husked, blemish-free #1 corms of Spic & Span gladiolus were disinfested with 0.26% sodium hypochlorite solution and each wounded with a sterile scalpel at three points along the lateral edge. Mycelia of C. trifolii from pure cultures were placed in the wounds of 20 corms. All corms were left in a moist atmosphere at room temperature for 8 days, during which the fungus became established in the wounded inoculated tissue. The corms were planted individually in thrice-autoclaved sandy soil in 4-inch plastic pots. When the developing foliage was 5 inches tall, the soil moisture was adjusted to about one-half of field capacity and potted plants were enclosed in polyethylene bags and placed at a constant temperature of 28°C.

After incubating for 20 days the corms were removed from the soil and disease progress noted. The soil in each pot was screened to remove roots. A second crop of two blemish-free #2 corms of Spic & Span was then planted in each pot and when foliage was 5 inches tall soil moisture was adjusted to about one-half of field capacity. Potted plants were then incubated in polyethylene bags at 28°C. Disease ratings for this second crop were made after 25 days' incubation.

Results: Wounded, uninoculated corms did not develop symptoms and the second control crop in the same soil remained healthy during the experiment.

Plants developing from wounded, inoculated corms had slight to severe root necrosis which was especially evident on roots just below corm spots, indicating that the fungus grew from the lesions into the soil and infected basal portions of roots. No internal connection between corm lesions and infected roots was evident. Some inoculated wounds enlarged during the period of soil incubation but corms did not rot completely.

Most plants of the second crop, grown in the same soil that supported the plants from artificially inoculated corms, were badly diseased. Root infection was the most common symptom,

while small corm lesions or general corm rot was found in 26 of the 40 corms planted. C. trifolii was isolated from 91% of the diseased plants of the second crop. The establishment of the pathogen in the soil by planting infected corms, and the persistence of the fungus during the crop periods, are evident from these results.

## DISCUSSION

The optimum temperature for growth in vitro of a corm isolate reported here confirms Van Weerdt's (8) findings and agrees with the range of McClellan and Marshall (4). Infection of foliage of the three varieties occurred from 24° to 36°C, a higher and smaller range than the 45° to 85°F range reported by McClellan and Marshall (4) for infection of Picardy foliage. The 24° to 36°C range substantiates field observations in Florida, although infection at 20° or slightly lower may occur. Infections of mature foliage from small corms of Florida Pink was slightly more severe than infection of foliage from large corms of Corona and Valeria. This relationship tends to confirm Magie's (3) observations.

The soils used in corm-root infection and soil infestation experiments were, like the soils cultivated by most Florida gladiolus growers, approximately 95 to 98% sand with little organic matter content. The persistence of C. trifolii in the soil for periods of up to 9 months has been demonstrated in experiments preliminary to this report.

The soil may become infested by 1) incorporation of infected plant debris, 2) planting infected corms, and 3) germination and growth of conidia that fall on the soil. From the results of the experiments where the soil became infested by planting inoculated corms and adding conidia to the soil surface, it seems probable that the fungus is able to grow, at least restrictedly, through the soil in the absence of gladiolus debris.

Establishment of C. trifolii in the soil by incorporating infected plant debris has been noted by many workers. There seems little reason to assume, however, that the fungus remains only as thick-walled hyphae or conidia in or on infected tissue as suggested by Mendiola-Ela (6). He (6) also reported that the fungus remains in the soil when infected corms rot. The present results show that corm rotting is not a necessary prerequisite since uniform soil infestation was obtained without enlargement of many corm lesions. Evidence of the possible role of cataphylls in the growth, parasitism, and spread of the fungus was not obtained, since cataphylls were removed from the corms used in these experiments. Cataphylls probably provide the fungus an important initial habitat for subsequent parasitism of the necks of the young plants and entry into the soil.

Evidence of the establishment of C. trifolii in the soil by means of conidia has not been reported previously. Adding an estimated 15,000 conidia to the soil surface of each 4-inch pot of soil resulted in severe infection of plants subsequently grown in the soil. Soil infestation by means of conidia has also been demonstrated using untreated field soil. An implication of these results is that the soils in or near gladiolus-growing areas, where Curvularia leafspot has occurred, may be infested.

Symptoms of root infection were frequently seen in plants incubated from 12° to 36°C. Root infection, often without corm infection, is common in the field. Discrete corm lesions developed over a range of 16° to 24°. At 28° and 32° lesions rapidly coalesced. In preliminary experiments, using a 36° incubation temperature, corm rot caused by Curvularia and bacteria was so rapid and severe that proper ratings could not be assigned. Leaf lesions that developed during incubation were clearly associated with the development of conidia on infected necks. Sporulation of C. trifolii on the soil surface was infrequent and could not be related definitely to leaf infection since, in all cases, sporulation on the necks was also present.

When the present information, reports from literature, and field observations are combined it is possible to diagrammatically represent some of the host-parasite relationships that are of interest to growers. Figure 1 indicates the pertinent cycles that probably occur in Florida. Neck infection of young plants growing in infested soil, although not shown in Figure 1 as occurring independently of corm or root infection, is commonly noted in the field and was found in the present investigations. Such infection may occur before corm or root symptoms appear.

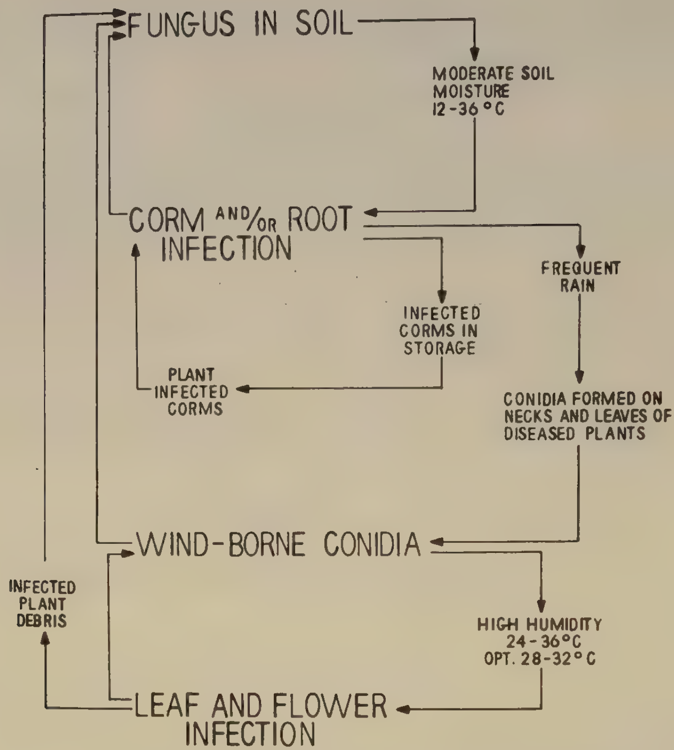


FIGURE 1. Diagram of some probable relationships of *Curvularia trifolii* to gladiolus and soil infestation in Florida.

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# INFLUENCE OF SPRINKLER IRRIGATION AND FOUR FORAGE CROPS ON POPULATIONS OF SOIL MICROORGANISMS INCLUDING THOSE ANTAGONISTIC TO SCLEROTIUM ROLFSII SACC.[1]

E. A. Curl

## Summary

The influence of sprinkler irrigation and four forage crops in effecting quantitative changes in microbial populations of soil was demonstrated in preliminary studies. While the principal factor influencing population changes appeared to be the kind of plant grown, the influence of irrigation also was evident. Fungi were slightly and consistently more abundant in plots of all crops receiving low-level irrigation. The largest number of fungi was obtained from fescue plots and the smallest number from alfalfa plots. The largest number of actinomycetes was found in fescue plots subjected to a high level of irrigation, while less than half this number was found in fescue plots of low-level irrigation. The highest populations of bacteria, including those antagonistic to Sclerotium rolfsii Sacc. in vitro, occurred in high-level irrigated white clover plots and the lowest populations in fescue plots receiving low-level irrigation.

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Microbial populations of the soil may be drastically changed both quantitatively and qualitatively by certain environmental factors. Important among these factors are soil moisture and kind of plant cover. During a screening program to find soil microorganisms that exhibit antagonistic effects upon root-infecting fungi, certain data were recorded relative to the effects of sprinkler irrigation and four forage crops on populations of fungi, actinomycetes, and bacteria.

## MATERIALS AND METHODS

The data were obtained from experimental plots on Greenville fine sandy loam soil at the Thorsby Foundation Seed Stocks Farm in Chilton County, Alabama. The experimental design and general procedures relating to the overall project were originally for the purpose of determining the adaptability of certain forage crops for irrigation (1). Subplots, each measuring 6 by 6 feet, were differentially irrigated by the system of movable sprinklers. Plots designated as receiving a low level of irrigation were given only enough water to assure survival of the plants, and were protected from natural rainfall by plastic-covered frames. Plots subjected to a high level of irrigation received both rainfall and irrigation water, which together amounted to almost three times the amount of water received by the low-level plots.

In September, 1 year after the plots were seeded, soil samples were taken with a soil sampling tube to a depth of 6 to 8 inches in the approximate rhizospheres of intermediate white clover, Kenland red clover, Atlantic alfalfa, and Kentucky 31 fescue. Composite samples were plated by the dilution plate method on three media: (a) peptone-dextrose agar with rose bengal and streptomycin sulfate for fungi, (b) glycerol-asparaginate agar for actinomycetes, and (c) Thornton's standardized medium for bacteria. Dilutions for the three groups of organisms were 1 in 10,000, 1 in 100,000 and 1 in 1,000,000, respectively. The procedures and the formulas of the culture media used were those described by Johnson, et al. (2). All cultures were incubated at temperatures ranging from 28° to 31° C. Fungal colonies were counted after 5 days of incubation and actinomycete and bacterial colonies after 10 days. Microbial isolates from randomly selected dilution plates were tested for antagonism to Sclerotium rolfsii Sacc. by applying the pathogen and contending organism simultaneously opposite each other on Czapek's sucrose-nitrate agar. Mycelial disks of the fungi were used in these tests, whereas actinomycetes and bacteria were streaked on the agar.

## RESULTS

Figures 1-3 show the average relative numbers of microoganisms occurring in soil cropped to the four forage crops under two levels of irrigation. Slightly and consistently more fungi were obtained from soil of each crop subjected to low-level irrigation than from soil of

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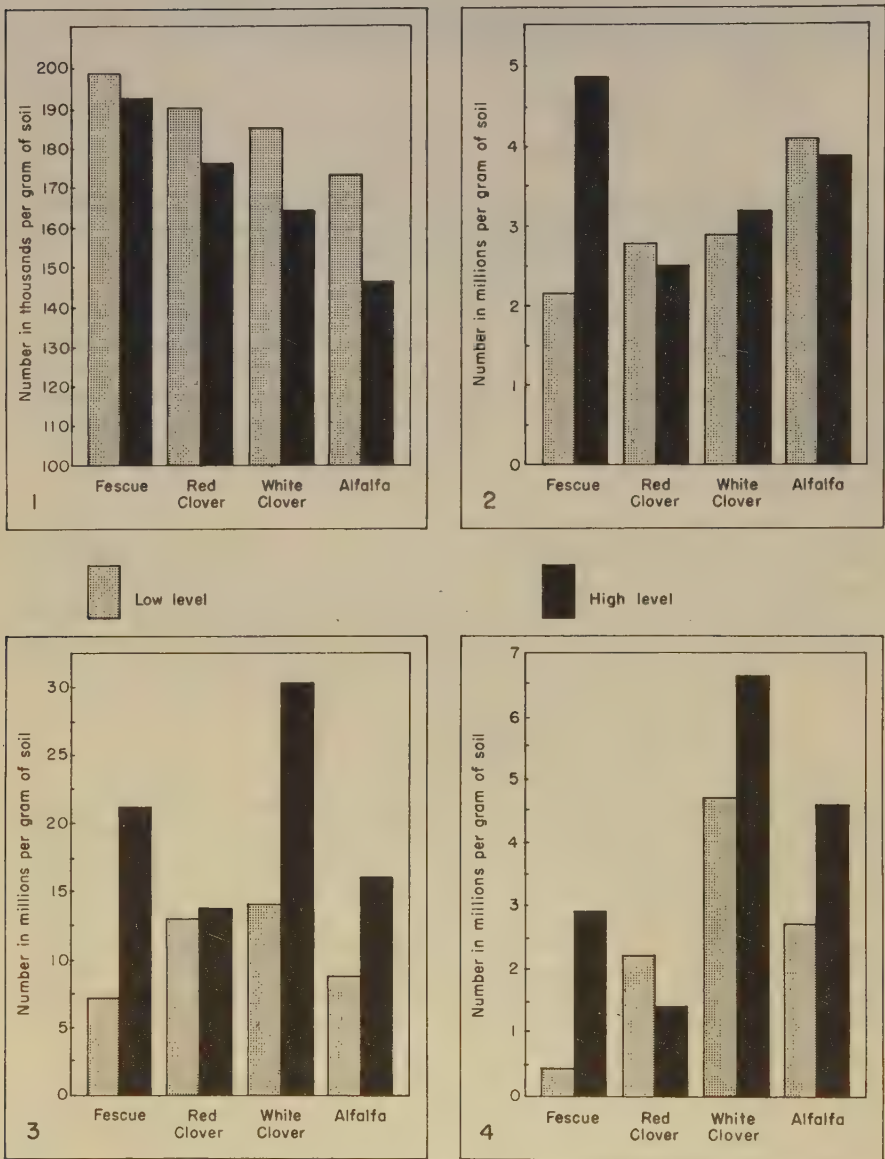
1 This work was done in connection with a project contributing to Southern Regional Project S-26.




FIGURES 1 - 4. Relative numbers of microorganisms in forage crops soil subjected to low and high levels of irrigation. 1 -- Fungi. 2 -- Actinomycetes. 3 -- Bacteria. 4 -- Bacteria antagonistic to *Sclerotium rolfsii*.

high-level irrigation (Fig. 1). The largest number occurred in fescue plots, and the smallest number in alfalfa plots. Of 506 fungal isolates tested, only *Trichoderma* was highly antagonistic to *S. rolfsii*. A number of others overran the pathogen and, thus, could be considered inhibitory.

With the exception of fescue plots, which had the greatest number of actinomycetes under high-level irrigation, there were practically no differences in numbers of this group between levels of irrigation for individual crops (Fig. 2). Alfalfa plots contained the next highest number of actinomycetes, the number being approximately equal for the two irrigation levels. One actinomycete of 652 isolates tested was strongly antagonistic to *S. rolfsii*.

The greatest differences in numbers of bacteria between levels of irrigation were found in white clover plots and in fescue plots, where high-level irrigation induced considerably more bacterial activity than under red clover and alfalfa (Fig. 3). Approximately 20% of the bacterial isolates from both irrigation levels of all crops exhibited some degree of antagonism to *S. rolfsii*. Most of these were obtained from highly irrigated white clover and alfalfa plots (Fig. 4).

## DISCUSSION

This preliminary work reemphasizes the highly erratic nature of microbial populations in the soil, since they are influenced by soil moisture and growing crop plants. Except for actinomycetes in fescue plots and bacteria in white clover, fescue, and alfalfa plots, population differences attributable to irrigation were negligible. Though soil samples processed in this study were not true rhizosphere samples, the sampling procedure unavoidably included much rhizosphere soil. The greater activity of microorganisms, particularly bacteria, in the rhizosphere of plants as compared with soil distant from roots has been repeatedly demonstrated (3, 5, 7, 8, 10, 11). Further, evidence has been presented (4, 6, 9) to show that this root zone may stimulate multiplication of certain organisms more than others depending upon species, age, and vigor of the crop plant grown.

The slight but consistently greater numbers of fungi found in plots of low-level irrigation might be the result from the natural tendency of certain fungi to sporulate more abundantly under environmental conditions that adversely affect vegetative growth. The predominant fungi isolated were the highly sporulating types, such as species of *Fusarium*, *Penicillium*, *Aspergillus* and *Trichoderma*. The selective influence of kind of crop plant on groups of microorganisms is indicated by the nearly inverse relation of populations of fungi and actinomycetes in Figures 1 and 2.

This work is far too preliminary to warrant definite conclusions regarding the value of specific crop plants or irrigation levels to effect control of *S. rolfsii* by natural microbial antagonism. However, the probable value of further studies along this line is indicated.

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# TOMATO RING SPOT VIRUS ISOLATED FROM EOLA RASP LEAF OF CHERRY IN OREGON[1]

J. A. Milbrath and J. E. Reynolds

Abstract

A rasp leaf disease of cherry new to Oregon was observed for the first time in August 1959. A virus was isolated from infected trees by sap inoculation to cucumber, petunia, tobacco, Chenopodium amaranticolor, and French bean. Serological and plant protection tests showed that the virus was related to tomato ring spot virus.

In August 1959 a virus-like rasp leaf disease of cherry was noted in the Eola Hills district of the Willamette Valley in a cherry orchard primarily of the Napoleon (Royal Ann) variety. Parts of the 119-acre orchard showed no evidence of the disease while in other areas 50% or more of the trees were dead or infected. The distribution pattern of the diseased trees was suggestive of a soil-transmitted virus.

Many of the large dying scaffold branches had numerous 1-year sprouts near the base. These sprouts had narrow to normal leaves with enations grouped along the midrib of the underside of the leaf. The small, narrow leaves produced by the early spring growth showed dense tomentose areas between the twisted distorted veins (Fig. 1).

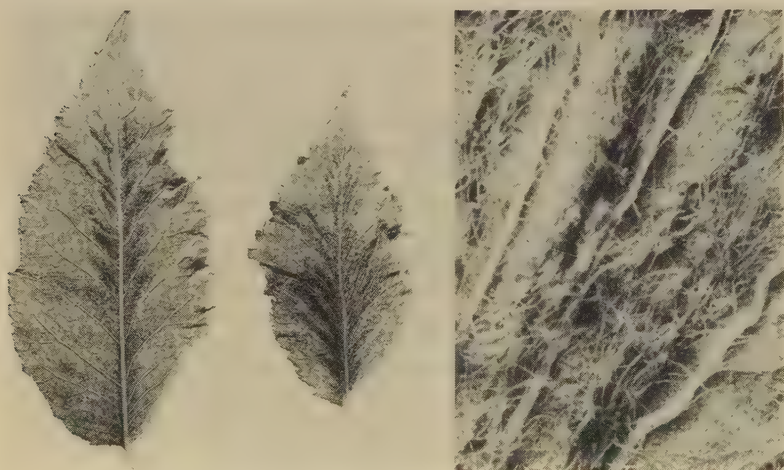

FIGURE 1. Small narrow leaves from the base of sucker growth on dying branches of Napoleon cherry trees infected with Eola rasp leaf. The detail photo from the smaller leaf shows the dense tomentose areas between the twisted distorted veins. (Photos by F. P. McWhorter)

This disease did not show the characteristic symptoms of any of the variants of rugose mosaic of cherry common in Oregon. Likewise, the die-back of the branches and the manner in which the disease spread in the tree were distinctive from that of cherry rasp leaf. The symptoms of the disease more closely resembled those illustrated by Mulder (4) for the Eckelradar disease in the Netherlands. This led to an early suggestion that the Oregon disease might be caused by the same virus or viruses as the Eckelrader disease or the Pfeffinger disease of cherry described by Blumer (1) in Switzerland.

A virus has been readily obtained by mechanical inoculations using juice from small immature terminal leaves or flower petals from infected orchard trees. This virus was also obtained readily from the young tip leaves of peach seedlings which had been inoculated with infected cherry buds the previous fall. The virus could be recovered by mechanical inoculations to cucumber, petunia, tobacco, Chenopodium amaranticolor or French bean, Phaseolus vulgaris. The ever-present stone fruit ring spot complex could be eliminated by using systemically infected French bean as a source of virus. Isolations from different trees in the orchard have varied greatly in their degree of severity on herbaceous hosts.

The symptoms produced by the virus on some herbaceous hosts are quite similar to those described for Arabis mosaic or raspberry ring spot. A. F. Posnette of the East Malling Station tested these isolates with antisera to Arabis mosaic and raspberry ring spot viruses. No serological relationships could be detected between these viruses and the Oregon isolates.

Dr. C. H. Cadman of the Scottish Horticultural Research Institute also expressed an interest in the Oregon virus as he had collected, studied, and prepared antisera to many of the soil-borne viruses. Two Oregon isolates designated as 100/3 and 105/1 were sent to him for a comparison with his collection of virus cultures. He reported by correspondence: "Both iso-

[1]Technical paper No. 1419, Oregon Agricultural Experiment Station.

lates 100/3 and 105/1 infected Chenopodium amaranticolor, Petunia hybrida and French bean (P. vulgaris var. Prince) systemically. Symptoms on all three hosts were typical of tomato ring spot virus but 105/1 usually produced only local lesions in petunia and rarely infected plants systemically. 105/1 protected Petunia plants from infection by the type (AC -13) and peach yellow bud strains of tomato ring spot virus, and clarified sap from inoculated P. hybrida leaves precipitated specifically with antiserum to peach yellow bud in tube tests. 105/1 was more difficult to work with but P. hybrida plants infected with 100/3 or with tomato ring spot virus were protected from infection by 105/1. Sap from plants with 105/1 gave a positive reaction with peach yellow bud antiserum in gel diffusion tests."


FIGURE 2. Lovell peach seedling snowing yellow bud condition the second season following fall inoculation with Eola rasp leaf.

The relationship of tomato ring spot virus and peach yellow bud virus was reported by Cadman and Lister (3). Peach yellow bud was first reported by Thomas and Rawlins in 1939 (5) as Winters mosaic, but was later renamed peach bud (6). Although cherry was mentioned in both articles as being infected with this virus, the symptoms described could have been caused by common latent viruses in stone fruits. In one experiment they reported that 1/7 cherry trees developed dwarfed distorted leaves 2 months after inoculation. The following spring the affected tree produced late appearing, small, pale leaves on shortened terminal growth. The yellow bud virus was recovered on peach from this tree.

Further evidence of the relationship of the Oregon virus to peach yellow bud virus was noted on greenhouse grown Lovell peach seedlings. These trees were dwarfed the next year after inoculation, the leaves were smaller than normal and dark-green colored. The leaves developed chlorotic blotches along the midrib and became twisted in a corkscrew-like manner. The following year the plants failed to develop normal leaves and exhibited only small yellow structures not more than a few millimeters long (Fig. 2). H. J. Jensen has recovered and identified Xiphenema americanum Cobb from the orchard and around infected trees. Proof of soil transmission with this nematode as a vector has not been attempted, but Breece and Hart (2) associated X. americanum with transmission of peach yellow bud mosaic.

The name Eola rasp leaf of cherry is proposed as the common name for this tomato ring spot induced disease. This name would be more definitive than peach yellow bud mosaic of cherry, and would relate this cherry disease to the other enation or rasp leaf type of diseases.

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# EFFECT OF SEED-TREATMENT FUNGICIDES ON GRAIN YIELD AND STANDS OF WINTER AND SPRING WHEAT[1]

C. R. Rohde and Laurence H. Purdy[2]

Summary

Eight chemical formulations commonly used as seed-treatment fungicides on wheat were studied to determine whether their use would affect the grain yield or stand of Omar winter wheat and Federation spring wheat in the absence of wheat bunt. These tests were carried out at the Pendleton Branch Experiment Station near Pendleton, Oregon from 1955 through 1959.

The best treatments resulted in yield increases in both Omar and Federation. Increases in stand in Omar tended to be less than those observed in Federation; however, the yield increases for both were approximately the same. Stand increases did not account for all the increase in yield, indicating that the fungicides might have had other effects on the wheat plants or organisms in the microenvironment of the wheat plant.

The fungicides and rates of application that resulted in the best stands and the highest grain yields were Panogen 15 at 3/8 and 3/4 ounce per bushel, Ceresan M at 1/2 ounce per bushel, Smut B Gon at 1/2 ounce per bushel, MEMA (liquid) at 1/4 ounce per bushel, and Anticarie at 1 ounce per bushel.

## INTRODUCTION

By 1957, hexachlorobenzene (HCB) had been generally accepted as the most effective seed-treatment fungicide for the control of common bunt (*Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro) of wheat in the Pacific Northwest. This fungicide was shown to be superior to all other fungicides tested as a result of its efficacy against both seed-borne and soil-borne common bunt (2). Prior to the use of HCB, the most commonly used seed-treatment fungicides were organic mercurials which were effective against seed-borne bunt only. However, the mercurials also control certain other wheat diseases of seed-borne origin. In contrast, HCB is specific for the wheat bunt fungi and to date has not been effective against organisms causing seedling diseases of wheat.

This study was initiated to compare the influence of HCB, a specific fungicide, with certain organic mercurials, non-specific fungicides, on seedling stand and grain yield of the wheat crop from fall and spring plantings in the absence of wheat bunt. These investigations were carried out at the Pendleton Branch Experiment Station, Pendleton, Oregon from 1955 through 1959.

## MATERIALS AND METHODS

The eight chemical formulations tested, along with their active ingredients and their manufacturers, are as follows[3].

Agrox -- 6.7% phenyl mercury urea. Chipman Chemical Company, Bound Brook, New Jersey.

Anticarie -- 40% hexachlorobenzene, H. P. Rossiger Company, Inc. New York.

Ceresan M -- 7.7% *N*-(ethylmercuri)-*p*-toluene sulfonanilide. E. I. duPont de Nemours and Co., Wilmington, Delaware.

Ceresan 75 -- 2.80% ethyl mercury 2,3-dihydroxypropyl mercaptide and 0.60% ethyl mercury acetate. E. I. duPont de Nemours and Co., Wilmington, Delaware.

[1] Cooperative investigations of the Oregon Agricultural Experiment Station, Pendleton and Crops Research Division, Agricultural Research Service, United States Department of Agriculture. Published with the approval of the Director of the Oregon Agricultural Experiment Station as Technical Paper No. 1406.

[2] Associate Agronomist, Pendleton Branch Experiment Station, Pendleton, Oregon, and Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Pullman, Washington.

[3] Mention of a trade name does not constitute a guarantee or warranty of the product named and does not signify that it is approved to the exclusion of other comparable products.

MEMA -- 11.4% 2-methoxy ethyl mercury acetate. Chipman Chemical Co., Bound Brook, New Jersey.
MEMA-SOL -- 23.85% methoxy ethyl mercury acetate. Chipman Chemical Co., Bound Brook, New Jersey.
Panogen 15 -- 2.2% cyano(methylmercuri)guanidine. Panogen Company, Division of Morton Chemical Co., Chicago, Illinois.
Smut B Gon -- 10% phenyl mercury acetate. California Spray-Chemical Corp., Richmond, California.

These formulations were applied to clean, bunt-free seed of Federation spring wheat during 1955 through 1959 and to Omar winter wheat during 1956 through 1959. The seed was treated in units of 250 ml with wettable powders applied as slurries and liquid formulations applied in a diluted form. To apply wettable powders at the 1/2-ounce rate, 120 mg of the formulation was placed in a vial with 2 ml of water and 2 to 4 drops of 5% methylcellulose added as a sticker. The contents of the vial were then made into a slurry and poured into a 1-liter Erlenmeyer flask containing the unit of seed to be treated. The fungicide was distributed over the seed surfaces by shaking and rotating the flask vigorously until all visible moisture had been removed from the inner wall of the flask. Higher or lower rates of application were made by increasing or decreasing proportionately the amount of fungicide used to make the slurry. An increase of about 10% of the calculated amount of fungicide needed to treat 250 ml of seed was used in the preparation of the slurry, to account for the amount of fungicide retained in the vial and on the inner wall of the flask.

Liquid fungicides applied at the 1/2-ounce rate were first diluted with water in a ratio of 1:16, and 2 ml of this dilution were used to treat one unit of seed. Higher or lower rates of application were made by altering the dilution ratio accordingly.

The fungicides were applied to seed at two rates: 1) the rate of application proved most effective for bunt control, 2) one-half the effective rate. The treated seed was kept in paper bags until planting.

The test plots were seeded with a 4-row V-belt seeder in rows 11 feet long. Grain yields and stand counts were made on 8-foot sections of the two center rows. Stand counts were made in the early spring on winter wheat plants in the 2- to 3-leaf stage of growth, and at a similar stage of growth in the spring wheat tests, usually 3 to 5 weeks after seeding.

Test plots were grown on summer-fallow land fertilized with 30 to 40 pounds of N per acre as ammonium nitrate. The data were analyzed by analysis of variance for the individual years and the treatments that were tested for the entire period of the experiment were analyzed by analysis of variance and analysis of covariance. Duncan's (1) multiple range test was used to compute significant differences in yield and stand.

## RESULTS AND DISCUSSION

Summaries of the grain yield and stand data obtained with Federation spring wheat are presented in Tables 1 and 2, respectively. The analyses of variances for the individual years indicated that in only 1 year out of 5 (1955) did the grain yields from the different seed treatments differ significantly. Furthermore, the analyses of variances on the stand data indicated that significant differences among stands of the different treatments occurred in only 2 of the 5 years. However, when the data for treatments that were tested for the entire 5-year period were analyzed statistically, significant differences were found between both grain yields and stands in the different treatments. Since stand differences were observed, the data were analyzed by analysis of covariance to determine whether yield differences observed were caused by differences in stand or were the result of other effects the fungicides might have on the ensuing crop. This analysis indicated that when the yields obtained from the different treatments were adjusted for differences in stand, the differences in yields were not statistically significant. Thus, the differences in yield among the treatments were largely due to differences in stand. However, a correlation coefficient of 0.64 was obtained between yield and stand, indicating that other factors were also causing differences in yield.

Wheat growers are interested in maximum yields of grain; therefore, treatments that resulted in increased grain yields of spring wheat should be used, provided the treatment selected is also effective in controlling bunt. Fungicides used in these studies that are effective against seed-borne bunt in the Pacific Northwest are Anticarie, Ceresan M, Ceresan 75, and Panogen 15 (2). These would be satisfactory for use on spring wheat. The results of this study are somewhat inconclusive for making recommendations, however, because although significant differences were observed among the treatments, the yield from check 1 was not sig-

Table 1. Mean yields of Federation spring wheat treated with different seed-treatment fungicides at the Pendleton Branch Experiment Station near Pendleton, Oregon, 1955-1959.

| Fungicide | Rate of fungicide (ounces per bu.) | Yield per acre (bu.) 1955 | 1956 | 1957 | 1958 | 1959 | Mean | Statistical significance[c] | % of checks | Adjusted yield[d] |
|---|---|---|---|---|---|---|---|---|---|---|
| Smut B Gon[a] | 1/2 | 30.5 | 49.0 | 50.2 | 14.4 | 62.1 | 41.2 | | 110 | 41.3 |
| Panogen 15 | 3/8 | 37.5 | 48.5 | 51.1 | 14.4 | 54.6 | 41.2 | | 110 | 40.4 |
| Ceresan M | 1/2 | 33.3 | 46.4 | 52.4 | 13.9 | 54.8 | 40.2 | | 107 | 39.5 |
| Anticarie | 1 | 25.9 | 49.9 | 50.6 | 12.8 | 61.2 | 40.1 | | 107 | 40.8 |
| Panogen 15 | 3/4 | 34.3 | 46.4 | 50.1 | 12.9 | 55.0 | 39.8 | | 106 | 39.0 |
| MEMA (liquid) | 1/4 | 30.8 | 47.6 | 49.2 | 12.9 | 57.1 | 39.6 | | 105 | 39.1 |
| Ceresan M | 1/4 | 30.0 | 46.4 | 49.1 | 14.2 | 56.2 | 39.2 | | 104 | 38.6 |
| Smut B Gon[a] | 1/4 | 28.4 | 46.5 | 50.3 | 12.9 | 56.1 | 38.8 | | 103 | 39.0 |
| Check 1 | | 25.2 | 47.6 | 49.1 | 13.1 | 56.2 | 38.2 | | 102 | 39.0 |
| Anticarie | 1/2 | 23.0 | 47.8 | 48.4 | 13.5 | 56.1 | 37.8 | | 100 | 38.3 |
| Check 2 | | 23.2 | 47.8 | 44.6 | 13.8 | 55.7 | 37.0 | | 98 | 38.1 |
| Agrox | 1/4 | 29.4 | 51.1 | | | | 40.2 | | 112 | |
| Agrox | 1/2 | 30.1 | 47.8 | | | | 39.0 | | 108 | |
| MEMA-SOL | 1/4 | 36.2 | | | | | 36.2 | | 150 | |
| MEMA-SOL | 1/2 | 33.3 | | | | | 33.3 | | 138 | |
| MEMA (liquid) | 1/2 | 32.0 | | | | | 32.0 | | 132 | |
| MEMA-SOL | 1/8 | | 48.1 | 52.2 | | | 50.2 | | 106 | |
| MEMA-SOL | 1/16 | | 46.2 | 51.1 | | | 48.6 | | 103 | |
| MEMA (liquid) | 1/8 | | 46.4 | 52.8 | 14.1 | 54.4 | 41.9 | | 102 | |
| Ceresan 75 | 3/4 | | | | 13.8 | 57.4 | 35.6 | | 103 | |
| Ceresan 75 | 3/8 | | | | 13.6 | 52.5 | 33.0 | | 95 | |
| Value of F[b] | | ** | N.S. | N.S. | N.S. | N.S. | * | | | N.S. |

[a] TAG was used in 1955 and Gytrete in 1956.
[b] N.S. not significant at 5% level; *significant at 5% level; **significant at 1% level.
[c] Duncan's multiple range test.
[d] Yields were adjusted on basis of differences in stand through the analysis of covariance.

Table 2. Mean stands of Federation spring wheat treated with different seed-treatment fungicides at the Pendleton Branch Experiment Station near Pendleton, Oregon, 1955-1959.

| Fungicide | Rate of fungicide (ounces per bu.) | Plants per plot (number) 1955 | 1956 | 1957 | 1958 | 1959 | Mean | Statistical significance[c] | % of checks |
|---|---|---|---|---|---|---|---|---|---|
| Panogen 15 | 3/4 | 228 | 298 | 246 | 119 | 200 | 218 | | 126 |
| Panogen 15 | 3/8 | 241 | 306 | 228 | 130 | 183 | 217 | | 126 |
| Ceresan M | 1/2 | 217 | 306 | 234 | 132 | 189 | 216 | | 125 |
| Ceresan M | 1/4 | 185 | 308 | 248 | 146 | 176 | 213 | | 123 |
| MEMA (liquid) | 1/4 | 177 | 303 | 245 | 136 | 189 | 210 | | 122 |
| Smut B Gon[a] | 1/2 | 138 | 307 | 199 | 136 | 198 | 196 | | 113 |
| Smut B Gon[a] | 1/4 | 138 | 321 | 190 | 132 | 194 | 193 | | 112 |
| Anticarie | 1/2 | 96 | 313 | 186 | 143 | 180 | 184 | | 106 |
| Anticarie | 1 | 86 | 316 | 187 | 134 | 178 | 180 | | 104 |
| Check 1 | | 108 | 296 | 166 | 131 | 182 | 176 | | 102 |
| Check 2 | | 124 | 278 | 144 | 135 | 164 | 169 | | 98 |
| Agrox | 1/2 | 176 | 305 | | | | 241 | | 119 |
| Agrox | 1/4 | 131 | 314 | | | | 222 | | 110 |
| MEMA-SOL | 1/2 | 209 | | | | | 209 | | 180 |
| MEMA-SOL | 1/4 | 204 | | | | | 204 | | 176 |
| MEMA-SOL | 1/2 | 194 | | | | | 194 | | 167 |
| MEMA-SOL | 1/8 | | 287 | 262 | | | 274 | | 124 |
| MEMA-SOL | 1/16 | | 297 | 217 | | | 257 | | 116 |
| MEMA (liquid) | 1/8 | | 304 | 254 | 137 | 174 | 217 | | 116 |
| Ceresan 75 | 3/8 | | | | 161 | 186 | 174 | | 114 |
| Ceresan 75 | 3/4 | | | | 129 | 196 | 162 | | 106 |
| Value of F[b] | | ** | N.S. | ** | N.S. | N.S. | ** | | |

[a] TAG was used in 1955 and Gytrete in 1956.
[b] N.S. not significant at 5% level; **significant at 1% level.
[c] Duncan's multiple range test.

Table 3. Mean yields of Omar winter wheat treated with different seed-treatment fungicides at the Pendleton Branch Experiment Station, near Pendleton, Oregon, 1956-1959.

| Fungicide | Rate of fungicide (ounces per bu.) | Yield per acre (bu.) 1956 | 1957 | 1958 | 1959 | Mean | Statistical significance[c] | % of checks | Adjusted yield[d] |
|---|---|---|---|---|---|---|---|---|---|
| Panogen 15 | 3/8 | 68.4 | 64.6 | 53.0 | 62.9 | 62.2 | | 109 | 61.9 |
| MEMA (liquid) | 1/4 | 65.4 | 62.0 | 52.7 | 68.9 | 62.2 | | 109 | 62.0 |
| MEMA (liquid) | 1/8 | 66.4 | 59.3 | 53.2 | 67.7 | 61.6 | | 107 | 61.7 |
| Smut B Gon[a] | 1/4 | 69.9 | 62.2 | 51.0 | 63.3 | 61.6 | | 107 | 61.6 |
| Ceresan M | 1/2 | 63.1 | 63.9 | 54.1 | 63.6 | 61.2 | | 107 | 61.0 |
| Panogen 15 | 3/4 | 65.2 | 63.2 | 51.0 | 63.6 | 60.8 | | 106 | 60.7 |
| Anticarie | 1 | 60.0 | 60.9 | 52.2 | 69.0 | 60.5 | | 106 | 60.6 |
| Smut B Gon[a] | 1/2 | 64.0 | 61.2 | 53.0 | 63.1 | 60.3 | | 105 | 60.4 |
| Anticarie | 1/2 | 59.3 | 56.4 | 54.6 | 68.5 | 59.7 | | 104 | 59.9 |
| Ceresan M | 1/4 | 66.2 | 59.3 | 54.5 | 55.0 | 58.8 | | 102 | 58.7 |
| Check 1 (no fungicide) | | 61.2 | 52.0 | 51.8 | 66.8 | 57.9 | | 101 | 57.9 |
| Check 2 (no fungicide) | | 60.5 | 56.8 | 52.6 | 57.2 | 56.8 | | 99 | 57.1 |
| MEMA-SOL | 1/16 | 64.8 | 60.7 | | | 62.8 | | 109 | |
| MEMA-SOL | 1/8 | 64.1 | 61.2 | | | 62.6 | | 109 | |
| Agrox | 1/4 | 66.0 | | | | 66.0 | | 108 | |
| Agrox | 1/2 | 63.1 | | | | 63.1 | | 104 | |
| Ceresan 75 | 3/4 | | | 51.1 | 67.8 | 59.4 | | 104 | |
| Ceresan 75 | 3/8 | | | 53.1 | 60.4 | 56.7 | | 99 | |
| Value of F[b] | | N.S. | N.S. | N.S. | * | * | | | * |

a Gytrete was used in 1956.
b N.S. not significant at 5% level; *significant at 5% level.
c Duncan's multiple range test.
d Yields were adjusted on basis of differences in stand through the analysis of covariance.

Table 4. Mean stands of Omar winter wheat treated with different seed-treatment fungicides at the Pendleton Branch Experiment Station near Pendleton, Oregon, 1956-1959.

| Fungicide | Rate of fungicide (ounces per bu.) | Plants per plot (number) 1956 | 1957 | 1958 | 1959 | Mean | Statistical significance[c] | % of checks |
|---|---|---|---|---|---|---|---|---|
| Panogen 15 | 3/8 | 238 | 198 | 196 | 188 | 205 | | 112 |
| Ceresan M | 1/2 | 240 | 197 | 166 | 203 | 202 | | 110 |
| MEMA (liquid) | 1/4 | 246 | 187 | 200 | 173 | 201 | | 110 |
| Panogen 15 | 3/4 | 234 | 191 | 168 | 186 | 195 | | 106 |
| Ceresan M | 1/4 | 237 | 182 | 153 | 205 | 194 | | 106 |
| Check 1 | | 225 | 137 | 200 | 193 | 189 | | 103 |
| Smut B Gon[a] | 1/4 | 239 | 170 | 168 | 175 | 188 | | 103 |
| Smut B Gon[a] | 1/2 | 228 | 172 | 174 | 177 | 188 | | 103 |
| MEMA (liquid) | 1/8 | 223 | 161 | 176 | 192 | 188 | | 103 |
| Anticarie | 1 | 246 | 138 | 166 | 184 | 184 | | 100 |
| Anticarie | 1/2 | 229 | 149 | 148 | 192 | 179 | | 98 |
| Check 2 | | 223 | 145 | 166 | 176 | 178 | | 97 |
| MEMA-SOL | 1/8 | 238 | 193 | | | 215 | | 118 |
| MEMA-SOL | 1/16 | 243 | 179 | | | 211 | | 115 |
| Agrox | 1/2 | 248 | | | | 248 | | 111 |
| Agrox | 1/4 | 236 | | | | 236 | | 105 |
| Ceresan 75 | 3/4 | | | 176 | 179 | 178 | | 97 |
| Ceresan 75 | 3/8 | | | 140 | 203 | 172 | | 94 |
| Value of F[b] | | N.S. | ** | ** | N.S. | ** | | |

a Gytrete was used in 1956.
b N.S. not significant at 5% level; **significant at 1% level.
c Duncan's multiple range test.

nificantly lower than that of the fungicides that gave the highest yields. On the other hand, the yield from check 2 was found to be significantly lower than the highest yielding treatments. There is a possibility that increased replication or further tests might show these differences to be real because the treatments that received no fungicide tended to rank lowest in yield.

Tables 3 and 4 present summaries of the grain yield and stand data, respectively, obtained with the different fungicides on Omar winter wheat. The analyses of variances for the individual years indicated that significant differences in grain yields from the different treatments occurred in only 1 of the 4 years; while significant differences in stands occurred in 2 of the 4 years. When the data for the 4-year period were analyzed statistically, the differences in both grain yield and stand were found to be significant. As with the spring wheat data, these data were analyzed by the analysis of covariance to determine whether the yield differences were the result of differences in stand or were caused by some other effect of the fungicide. The analysis of covariance indicated that differences existed among the yields from the different treatments even after adjustments were made for differences in stand. The correlation coefficient between yield and stand was 0.62. These results suggest that in addition to increasing stands, some of the fungicides might have had other beneficial effects on the wheat plants which, combined with increased stands, resulted in increased grain yields. Possibly these fungicides controlled other seedling diseases that only weakened the plants and reduced grain yields of plants grown from untreated seed. This conclusion tends to be substantiated by the rather low correlation coefficients, $r = 0.64$ for the spring wheat and $r = 0.62$ for the winter wheat, which were observed between stand and grain yields. If stands were the primary factors that determined the increased yields observed in this study, then the correlation coefficients should have been higher.

In a comparison of the two experiments, the magnitude of the increases in yields was very similar for winter and spring wheat. The best treatments resulted in grain yields that averaged 5 to 10% higher than yields from untreated seed. These treatments were Panogen 15 at 3/8 or 3/4 ounce per bushel, Ceresan M at 1/2 ounce per bushel, Smut B Gon at 1/2 ounce per bushel, MEMA (liquid) at 1/4 ounce per bushel, and Anticarie at 1 ounce per bushel. These data suggest further that Anticarie (HCB), which is believed to be specific for wheat bunt, might adversely influence other disease-producing organisms; or that HCB might favorably affect the host plant. Furthermore, Anticarie and other fungicides whose primary active ingredient is hexachlorobenzene, when applied to seed, are the only formulations that effectively control bunt that originates from soil-borne spores. Therefore, these are the only fungicides effective for bunt control in winter wheat where soil-borne spores of bunt occur.

These tests also indicated that the favorable effect of the fungicides on stand was somewhat greater on Federation spring wheat than on Omar winter wheat. Stand increases in spring wheat ranged from 13 to 26%, whereas in winter wheat these increases ranged from 3 to 12%.

## Literature Cited

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# LEAF DROP OF TAXUS[1]

Irene H. Stuckey[2]

Abstract

Lesions on the under surface of the leaves of Taxus from which Alternaria has been isolated appear to be related to the excessive abscission of leaves that is becoming increasingly frequent in nurseries and established plantings of Taxus in Rhode Island.

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Leaf drop of Taxus in excess of the normal annual abscission at the time of new growth in June has been an increasing problem in nurseries and in established plantings in Rhode Island for the past ten years. The leaves turn bright yellow beginning at the base and shed up to and sometimes including the current year's growth, leaving bare twigs. This excessive abscission was first noticed on Taxus cuspidata nana, but more recently it has been observed on other varieties of T. cuspidata and on T. media which is a hybrid between T. cuspidata and T. baccata (Fig. 1). In June 1960, abscission of the leaves of T. cuspidata capitata was especially severe. Trees up to 5 feet tall lost all the leaves on the lower one-third to one-half of their branches and the branches died.

Since pathologists could isolate no organisms and since many of the affected plants tended to recover after being transplanted to a different location, the problem was thought to be physiological and research was begun on that basis.

A series of experiments with plants of various ages in the greenhouse and in the field investigated water relations, pH of the soil, photoperiod and its relation to dormancy, nutrition, and cultural treatments including mulches and root pruning. All gave negative results for the leaf drop in question.

However, during these investigations lesions were noticed on the underside of some of the leaves. These developed on new leaves between July 20 and August 1. A few of the severely spotted leaves turned yellow and dropped in October and November, particularly those on the long shoot growth, but most abscised during the period of rapid growth between June 1 and June 15. These leaves would abscise whether they were in the age-class that would normally be shed at that time or not. In late July when the current year's growth had matured, lesions appeared on these leaves and the cycle began again. Plants that had been root-pruned early in May, and plants growing in unfavorable situations, lost a higher percentage of leaves (and sometimes young twigs) than young plants growing in favorable situations, but even young plants lost an abnormally large number of leaves when the leaves were severely spotted.

The lesions began as small raised areas, round or oval, on the under surface of the leaves. At first these raised areas were green, then as the cells involved died, they became chocolate brown with a small pit-like hole in the center (Figs. 2, 3). When dissected, the lesions were found to be formed from mesophyll cells that had increased to four times normal size. Palisade cells did not appear to be affected. Occasionally a two-celled spore, pointed at the ends, could be found.

Further evidence of the relation of the chocolate-colored lesions and leaf drop was shown when 7-year-old plants of several varieties of Taxus were transplanted into the field in November 1958. These plants had the chocolate-colored lesions on their leaves and were so badly defoliated that only the current year's growth contained leaves. They were placed next to a block of plants with several cultural treatments that had been isolated from other Taxus plants for 3 years and had had no leaf spot and no excessive abscission. By late July of the following year, 1959, the newly matured leaves showed the same lesions and the spotted leaves were shed in June 1960. In this particular case, the older plants did not recover and were barely alive when removed from the field on April 28, 1961. The leaves of the young plants were heavily spotted during 1960 and 1961 but these plants were also removed from the field on April 28 before new growth had begun.

Cultures from these lesions made in another laboratory in late February and early March 1960 from three varieties of Taxus gave Alternaria sp. from each lesion and nothing else. Cultures made from cankers on some of the twigs produced Pestalotia sp., but where cankers

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FIGURE 1. Yellowing and subsequent shedding of leaves of three varieties of Taxus. A -- Taxus cuspidata nana, B -- T. cuspidata densiformis, C -- T. media hicksii.

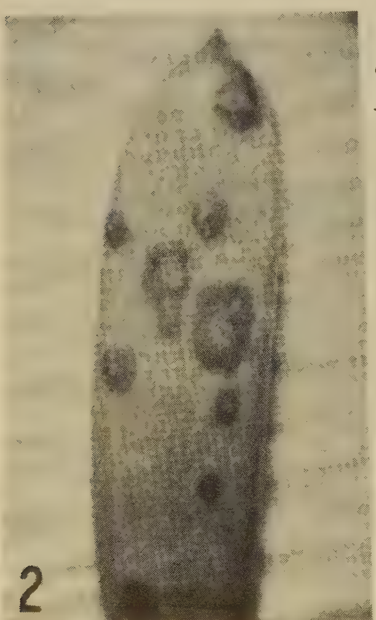


FIGURE 2. Lesions found on underside of leaf of Taxus cuspidata nana.

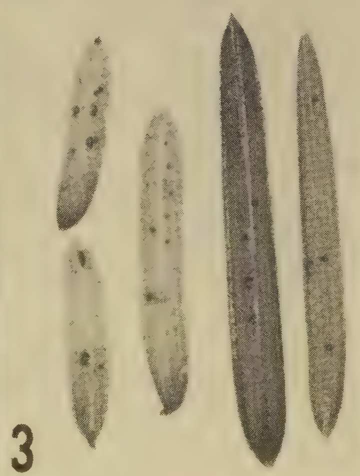


FIGURE 3. Lesions on single leaves of four varieties of Taxus, from left to right as follows: T. caspidata nana, T. cuspidata spreading, T. media hatfieldii, T. baccata repandens.

occurred leaves became bright brown and not yellow and remained attached to the twigs.

While no particular survey was made in other States, Taxus plants were examined at every opportunity. Lesions of the same type accompanied by excessive defoliation were found on the underside of leaves of Taxus, especially Taxus cuspidata nana, in Massachusetts and Connecticut and at one location near New York City. None of the plants examined in Pennsylvania had spots on the leaves or excessive abscission.

In Rhode Island, lesions of the type described have been found on all varieties of Taxus examined, including T. baccata repandens and T. media hatfieldii (Fig. 4), but the degree of abnormal shedding associated with these lesions varied from year to year. The range was from



FIGURE 4. Lesions but no defoliation on A -- *Taxus media hatfieldii*, B -- *T. baccata repandens*.



FIGURE 5. Lesions and defoliation on A -- *Taxus cuspidata nana*, B -- *T. cuspidata* spreading.

none on the particular plants of *T. baccata repandens* examined to severe in *T. cuspidata nana* and *T. cuspidata capitata*, with the other varieties falling in between (Fig. 5). However, in every instance where the leaves turned bright yellow and shed in excess of the normal shedding, the characteristic lesions appeared on the leaves.

Since excessive defoliation of *Taxus* appears related to the presence of chocolate-colored lesions found on most of the leaves that are shed, pathogenicity of *Alternaria*, the fungus found in these lesions, should be studied.

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# TENTATIVE INSECT VECTORS OF CLAVICEPS PASPALI OF DALLIS GRASS IN MISSISSIPPI

G. K. Parris[1] and Bobby Moore[2]


## Abstract

Twenty-five insects are tentatively identified as possible vectors of Claviceps paspali in Mississippi. The literature on this relatively poorly investigated subject is reviewed and evaluated from an international standpoint.


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Claviceps paspali F. L. Stevens & J. G. Hall is common on the campus of Mississippi State University and the adjacent roadsides of Starkville, Mississippi every summer and fall, where Dallis grass (Paspalum dilatatum) forms part of the natural grass cover. A survey was made from September 1959 through September 1960 of the insects visiting the sphacelial stage, whose ooze contains asexual conidia, the secondary inoculum, by means of which the pathogen is disseminated, chiefly by said insects. Ascospores, the primary inoculum, are believed to be disseminated chiefly by wind, although in the past some have not agreed. Professor Peter Henry Rolfs of Clemson University, cited by Stevens and Hall (6), thought that beetles, mostly of the family Carabidae, were ascospore vectors, and Stäger (5) reported that he observed many flies visiting the sphaeridia (stromatal heads containing the perithecia) of the germinating sclerotia. Rolfs wrote to Stevens and Hall that he noted the beetles running over the ground where sclerotia were producing ascospores. The beetles, seeking a high point from which to fly, would run up the culms of the Dallis grass, over the pistils and thus bring about inoculation of the ovaries and subsequent infection.

Not much work has been done on the vectors of C. paspali and the present short paper is an attempt (a) to summarize what has been reported previously, and (b) to report local findings, comparing them with national and international results.

The first reference of significance is that of Atanasoff (1) who in 1920 wrote a long discourse on the known facts about all the Claviceps species that cause ergot of grains and grasses. Atanasoff's work is unpublished, and this fact seems to have kept even Leach (4) from including details of the 40 different insects that Atanasoff reported as feeding upon the exudate of ergot, caused by various Claviceps species, namely: C. purpurea (Fr.) Tul., C. balansioides Moell., C. caricina Griff. & Morris, C. cinerea Griff., C. junci Adams, C. lutea Moell., C. microcephala (Wallr.) Tul., C. nigricans Tul., C. pallida var. orthocladea P. Henn., C. paspali, C. philippii Rehm, C. pusilla Ces., C. ranunculoides Moell., C. rolfsii Stev. & Hall, C. sesleriae Stäger, C. setulosa (Quél.) Sacc., C. tripsaci Stevens & Hall, C. wilsoni Cooke, and C. spp. (one on Spartina stricta, the other on Zizania aquatica and Z. palustris). Through the kindness of Dr. C. R. Benjamin, Principal Mycologist, National Fungus Collections, Plant Industry Station, Beltsville, Maryland, the senior author was permitted to examine a copy of Atanasoff's mimeographed unnumbered publication. In Doctor Benjamin's words, "This publication is rare and this is our only copy." (Letter of April 24, 1961).

None of the 40 insect vectors reported by Atanasoff was original with him. He says, "The following is a list of insects reported (by various workers, whose names he lists) that help in the dissemination of Claviceps ... It is supposed that what is written of C. purpurea is true in a general way also of the other species of the same genus, although ... there is, in the majority of cases, still no supporting experimental evidence."

The insects reported by Atanasoff were divided by him into three types, (a) "pollen-eating insects," (b) "honeydew sucking insects," or (c) neither type (a) nor type (b); they are arranged below, classified into Orders and Families by the writers. Assistance here was kindly given by Mr. Calvin R. Andress, Assistant Entomologist, State Plant Board, Stage College, Mississippi. The "pollen-eating" type is considered to be similar to vectors that carry inoculum externally (Langdon and Champ, 3), and the "honeydew sucking" type is thought comparable to Langdon and Champ's vectors that ingest conidia, carry them in the gut, and pass them in the faeces unharmed.

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**Conidia Carried Externally**

- Diptera
  - Anthomyidae
    - An unidentified genus
    - *Ophyra anthrax* Meig.
  - Lonchaeidae
    - *Lonchaea* (*fumosa* ?)
  - Syrphidae
    - *Melanostoma mellina*
    - *Melithreptus mentastri*
  - Lauxaniidae
    - *Sapromyza quadripunctata*
  - Sarcophagidae
    - *Sarcopha* (sic) = *Sarcophaga nigriventris* or *S. depressiformis*

**Conidia Carried Internally**

- Diptera
  - Anthomyidae
    - An unidentified genus
    - *Ophyra anthrax* Meig.
  - Lonchaeidae
    - *Lonchaea fumosa* (?)
  - Syrphidae
    - *Melanostoma mellina*
    - *Melithreptus mentastri*
    - *Pipicella* (sic) = *Pipizella vespillo*
    - *Platycheirus peltatus*
  - Lauxaniidae
    - *Sapromyza quadripunctata*
  - Sarcophagidae
    - *Sarcophaga nigriventris* or *S. depressiformis*
  - Calliphoridae
    - *Lucilia sylvarum*
    - *Pollenia vespillo*
  - Fungivoridae
    - *Sciara* sp.
- Hemiptera
  - Scutelleridae
    - *Eurygaster maura*
  - Miridae
    - *Miris holsatus*
- Hymenoptera
  - Vespidae
    - *Vespa* sp.
  - Ichneumonidae
    - *Amblytheles subsericans*
  - Formicidae
    - Various ants
  - Apidae
    - *Apis mellifica*
- Coleoptera
  - Coccinellidae
    - *Coccinella quinquepunctata*
    - *C. septempunctata*
  - Cantharidae
    - *Rhagonycha fulva*
- Identity uncertain:
  - *Doliophus vulgaris*
  - *Pompylus viaticus*

**Conidial Lodgment Not Specified**

- Diptera
  - Anthomyidae
    - *Hylemyia* sp.
  - Syrphidae
    - *Cheilosia* sp.
  - Lauxaniidae
    - *Sapromyza apicalis*
    - *Sapromyza* sp.
  - Fungivoridae
    - *Sciara thomae* L.
  - Tachinidae
    - *Tachina* (?) sp.
  - Rhagionidae
    - *Leptis tringaria* L.
  - Sarcophagidae
    - *Sarcophaga* sp.
  - Tetranoceridae
    - *Tetranocera ferruginea*
- Hymenoptera
  - Vespidae (?)
    - Wasp (sic)
  - Magachillidae (?)
    - Leaf wasps
  - Ichneumonidae
    - *Lissonata cylindrator* L.
  - Sphecidae
    - *Mimesa dahlbomi*
- Coleoptera
  - Cantharidae
    - *Podabrus alpinus*
    - *Cantharis melamura*
- Identity uncertain:
  - *Anustronyche abdominalis* Fbr.
  - *Brachytropsis calaratus*
  - *Dolorus* (sic) *pratensis* (*Dolerus* ?)
  - *Egeria pararge*
  - *Tropicoris rufipes*

Atanasoff concludes, "Of all these insects, only *Melanostoma mellina* and *Rhagonycha fulva* are commonly found on a number of plants, while the others are common only on certain plants and are considered less important in the spreading of *Claviceps* than the first two."

In 1954, Langdon (2) reported that a metallic green fly, *Pyrellia coerulea* (sic) was the chief vector of *Claviceps paspali* in Queensland, Australia. Later, Langdon and Champ (3) found *Pyrellia caerulea* was not only the most abundant vector, but that this insect ingested the conidia which it then passed in the faeces, unharmed. In careful studies, these workers, one a plant pathologist, the other an entomologist, classified approximately 28 insects that either ingested conidia or

carried them externally. The former were characterized as "strong flying insects, predominant among which is Pyrellia caerulea", the latter as "chance visitors," for example larger Orthoptera and Arachrida, thrips and aphids. Their list is given verbatim as follows:

Conidia in Gut

Diptera
- Musciinae
  - Pyrellia caerulea Wied.
- Phaoniinae (sp. indet.)
- Fanniinae (sp. indet.)
- Calliphoriinae (sp. indet.)
- Sarcophagiinae (sp. indet.)
- Tachiniinae (sp. indet.)
- Lauxaniidae (sp. indet.)
- Sepsidae (sp. indet.)
- Tetanoceridae (sp. indet.)

Hymenoptera
- Vespidae
  - Rhopalidia grejaria Sauss.
- Ichneumonidae
  - Paniscus spp.
  - Lissopimpla semipunctata Kby.
- Braconidae (sp. indet.)

Orthoptera
- Tettigoniidae (sp. indet.)

Lepidoptera
- Crambidae (sp. indet.)

Conidia Carried Externally

Coleoptera
- Chrysomelidae
- Coccinellidae
- Phalacridae

Hemiptera
- Jassidae
- Membracidae
- Cixiidae
- Pentatomidae (nymphs only)
- Aphididae

Orthoptera
- Blattidae
- Acridiidae

At Mississippi State University and around Starkville, insects were collected by taking random sweeps with a net, at various daylight hours, in and around diseased Dallis grass inflorescences. The insects captured were chloroformed and identified. The list is given below. There seemed to be no one insect or insects more predominant than others, and there likewise was no apparent difference in collections made at different daylight hours. Insects could be found feeding, or visiting plants, at all hours. However, it was noted that the smaller insects, such as ants, aphids, fungus gnats and the like, like to feed on relatively warm, cloudy days, whereas the larger insects, such as beetles and flies, favor hotter, sunny days. As expected, moist conidial ooze was most prevalent in the early morning or after sundown except on rainy days when it was present for a considerable time. As the air dried and the temperature rose, the ooze also dried, but insects, probably attracted by its carrionlike odor (4), seem to visit it anyhow. No data were obtained on the presence of Claviceps conidia either within or upon the bodies of the insects listed. Therefore, their classification as vectors should be considered to be tentative only; the circumstantial evidence is strong, however, no weaker than that given by Atanasoff (1), but certainly not the measure of that presented by Langdon and Champ (3).

Diptera
- Musca domestica
- Muscina stabulans
- Muscina sp.
- Sarcophaga sp.
- Limnia sp.
- Callitroga macellaria
- Various spp. of Fungivoridae

Coleoptera
- Diabrotica undecimpunctata howardi
- Hippodamia convergens
- Adalia bipunctata
- Various spp. of Chrysomelidae

Hemiptera
- Solubea pugnax
- Various spp. of Lygaeidae

Homoptera
- Draeculacephala portola
- Oncometopia undata
- Lepyronia quadrangularis
- Various spp. of Aphididae

Hymenoptera
- Monomorium minimum
- Crematogaster lineolata
- Paratrechina sp.
- One unidentified species of Braconidae
- One unidentified species of Ichneumonidae

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# COMPARATIVE EFFECTIVENESS OF VARIOUS NEMATOCIDES IN THE CONTROL OF ROOT KNOT IN MUCK SOIL

J. D. Wilson[1]

## Summary

Twenty nematocides were mixed with muck soil heavily infested with the root-knot nematode Meloidogyne hapla in a comparison of their ability to prevent the infestation of the roots of tomato plants. At the application rates chosen for the different chemicals EDB gave the best control, followed in effectiveness by MC-2, DBCP, and Cynem (EN-18133). Vapam, Mylone, EP-162, Bedrench, and EN-28450 ranked next in that order. Chloropicrin, formaldehyde, allyl alcohol, and phorate (Thimet) gave comparatively poor control.

In a comparison of a water seal and a polyethylene tarp for confining the nematocides in the treated soil, the latter method was more effective in reducing the number of Meloidogyne larvae and in checking infestation of the tomato roots. Plant growth and fruit production was better when the soil was tarped, but was also greater in the untreated checks than in the average of all treated plots. The average root weight was greater with heavy root-knot infestation than where few knots were present.

Good root-knot control, with low populations of Meloidogyne larvae at the end of the experiment, was accompanied by high populations of free-living forms of nematodes and, conversely, the number of free-living nematodes present was low when root-knot control was poor.

## INTRODUCTION

The control of nematodes by chemical means in muck soil, with its high adsorptive and absorptive capacities for liquids and gases, is much more difficult than in mineral soils that hold less water and may contain more air per unit of soil volume. This inherent difficulty is usually compensated for by the recommendation that the application rate considered to be optimum for mineral soils be increased by 50 to 100% when muck is to be treated.

Previous experience by various investigators, including the author (2, 3, 4), has determined, within rather narrow limits, the application rate for a number of compounds that will provide an acceptable degree of control of the northern root-knot nematode (Meloidogyne hapla) in the muck soil of the northeastern portion of the United States. However, since the list of chemicals with nematocidal properties has been increasing during the past few years, it was decided to compare several of those now available in a single experiment. Also, because of the recent interest in the possible use of polyethylene film to slow down the rate of escape of the gases given off by the more volatile nematocides, and thus hold them in more intimate contact with the soil over longer periods of time, provision was made to "tarp" twelve of the compounds included in the experiment.

## METHODS

On November 3, 1960, twenty-two nematocidal candidates[2], some on the market and time-tested and others experimentally new, were each mixed with muck soil known to be heavily infested with the root-knot nematode, by placing the chemical and the soil, in lots of 2 cubic feet each, in a rotating cement mixer for a period of 3 minutes. The treated lots of soil were then placed in separate compartments, each with an area of 5 square feet, in benches in a greenhouse at the Muck Crops Substation at Celeryville, Ohio. All lots of soil not confined in polyethylene covers were immediately "sealed" with water to a depth of about 1 inch of soil. The polyethylene covers were removed from the tarped compartments after 3 days and the soil watered in the same way as the others. Two weeks after treatments had been applied, ten Tiny Tim tomato plants (a variety that reaches a maximum size of about 12 inches in height and breadth and has fruits about 1/2 inch in diameter) were set in the soil of each compartment. The compounds being compared, and the rates at which they were applied, are indicated in Table 1. Two compartments were filled with untreated soil and one with soil that

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[2]Chemical formulations are listed on page 536.

Table 1. Effect of various nematocides on the number of M. hapla larvae that can be recovered from treated soil, and on the degree of root-knot development on tomato plants. Some lots of treated soil were sealed with water and others with a polyethylene tarpaulin.

| Treatment | Rate/acre in gallons or pounds | Tarped yes or no | Nematodes per pint of soil: free-living forms | Nematodes per pint of soil: Meloidogyne larvae | Other plant parasitic nematodes | Root-knot score[a], none = 0, maximum infestation = 4: 0 | 1 | 2 | 3 | 4 | Averages |
|---|---|---|---|---|---|---|---|---|---|---|---|
| EDB (W-85) | 15 gal. | no | 2491 | 53 | | 10 | | | | | 0.0 |
| do. | 15 gal. | yes | 5406 | 0 | | 10 | | | | | 0.0 |
| Fumazone (70-E) | 8 gal. | no | 3551 | 0 | | 9 | 1 | | | | 0.1 |
| do. | 10 gal. | no | 4982 | 212 | | 7 | 3 | | | | 0.3 |
| do. | 10 gal. | yes | 10176 | 0 | | 10 | | | | | 0.0 |
| do. | 12 gal. | no | 6095 | 0 | | 10 | | | | | 0.0 |
| Nemagon (EC-2) | 10 gal. | no | 5141 | 0 | Pra. | 6 | 4 | | | | 0.4 |
| do. | 10 gal. | yes | 6042 | 0 | Pra. | 8 | 2 | | | | 0.2 |
| Nemagon granules (17.3%) | 500 lb. | no | 3392 | 53 | | 9 | 1 | | | | 0.1 |
| EP-162 | 100 gal. | no | 3710 | 371 | | 5 | 4 | 1 | | | 0.6 |
| do. | 100 gal. | yes | 3975 | 0 | Pra. | 9 | 1 | | | | 0.1 |
| Vapam | 60 gal. | no | 3869 | 0 | Pra. | 6 | 4 | | | | 0.4 |
| do. | 60 gal. | yes | 9804 | 0 | | 9 | 1 | | | | 0.1 |
| Mylone (85-W) | 250 lb. | no | 6837 | 53 | Para. | 9 | 1 | | | | 0.1 |
| MC-2 | 1 lb./100 sq. ft. | yes | 7420 | 53 | | 8 | 2 | | | | 0.2 |
| do. | 2 lb./100 sq. ft. | yes | 4399 | 0 | | 10 | | | | | 0.0 |
| Steam | 185° F/2 hrs. | yes | 4399 | 0 | | 10 | | | | | 0.0 |
| EN-28450 | 20 gal. | no | 10971 | 106 | Pra. | 8 | 2 | | | | 0.2 |
| Cynem | 6 gal. | no | 4982 | 53 | | 10 | | | | | 0.0 |
| do. | 6 gal. | yes | 2438 | 0 | | 3 | 7 | | | | 0.7 |
| Cynem (5% granules) | 500 lb. | no | 2650 | 0 | Pra. | 9 | 1 | | | | 0.1 |
| do. | 500 lb. | yes | 13303 | 636 | Pra. | 7 | 3 | | | | 0.3 |
| Formaldehyde | 150 gal. | no | 2438 | 10335 | | | 2 | 5 | 3 | | 2.1 |
| do. | 150 gal. | yes | 5149 | 1643 | | | 5 | 5 | | | 1.5 |
| Telone | 40 gal. | no | 6519 | 159 | | 4 | 6 | | | | 0.6 |
| do. | 40 gal. | yes | 6837 | 53 | | 6 | 4 | | | | 0.4 |
| D-D | 40 gal. | no | 6360 | 1378 | | 1 | 8 | 1 | | | 1.0 |
| Chloropicrin | 40 gal. | no | 4558 | 5353 | Pra. | | | 6 | 4 | | 2.4 |
| do. | 40 gal. | yes | 11395 | 42 | Para. | | 8 | 2 | | | 1.2 |
| Allyl alcohol | 100 gal. | no | 2756 | 4505 | Pra. | | | 7 | 2 | 1 | 2.4 |
| do. | 100 gal. | yes | 5618 | 530 | | | 4 | 5 | 1 | | 1.7 |
| Phorate (4 lb./gal.) | 5 gal. | no | 1802 | 4021 | | | 3 | 5 | 2 | | 1.9 |
| do. | 5 gal. | yes | 2862 | 1749 | | | 3 | 7 | | | 1.7 |
| Dorlone | 25 gal. | no | 5512 | 10476 | | | 8 | 2 | | | 1.2 |
| Bedrench | 75 gal. | no | 6572 | 1431 | | 5 | 5 | | | | 0.5 |
| A-A + D-D | 75 gal. | no | 2862 | 8720 | | 1 | 8 | 1 | | | 1.0 |
| CC-9882 | 6 gal. | no | 6413 | 6837 | | | 6 | 3 | 1 | | 1.5 |
| N-dure | 150 gal. | no | 3286 | 8190 | Pra., Para. | | 1 | 5 | 2 | 2 | 2.5 |
| C-1000 | 25 gal. | no | 3445 | 3180 | Pra. | | 2 | 2 | 4 | 2 | 2.6 |
| SD-4741 | 10 gal. | no | 3445 | 7120 | | | | 4 | 5 | 1 | 2.7 |
| Check | | no | 6625 | 3816 | | | 2 | 5 | -3 | | 2.1 |
| Check | | no | 6996 | 5883 | | | 3 | 5 | 2 | | 1.9 |
| Averages of various treatment groupings: | | | | | | | | | | | |
| 12 that were tarped | | | 6837 | 445 | | 5.2 | 3.2 | 1.6 | 0.1 | 0.0 | 0.66 |
| Same 12 not tarped | | | 2332 | 4028 | | 4.8 | 2.3 | 2.0 | 0.9 | 0.1 | 0.93 |
| 12 of very heavy infestation | | | 4544 | 5242 | | 0.0 | 3.5 | 4.6 | 1.8 | 0.3 | 1.88 |
| 12 of very light infestation | | | 5285 | 93 | | 7.6 | 2.3 | 0.1 | 0.0 | 0.0 | 0.25 |
| 2 untreated checks | | | 4850 | 6837 | | 0.0 | 2.5 | 5.0 | 2.5 | 0.0 | 2.00 |

Pra. = Pratylenchus spp. present in small numbers. Para. = Paratylenchus spp. present in small numbers.

[a]Root-knot score: Zero (0) corresponds to no root knot, and four (4) indicates the maximum degree of infestation present in the experiment.

had been treated with steam in an autoclave. In one instance a single material was applied at three rates. Two compounds were applied as both liquid and granular formulations, and a total of 12 treatments were compared in tarped and untarped lots of soil.

The experiment was concluded 117 days later, at which time the tomato plants were removed from the soil. The root system of each plant was examined and scored according to the degree of root-knot infestation present. Soil samples were also taken from each compartment, and these samples then were subjected to a modified Baermann funnel technique (1) to determine the number of Meloidogyne larvae present per pint of soil. Other data that were taken when the plants were removed from the differently treated plots included the weights of the tops and the root systems and of the fruits present on the plants at the time of harvest, none having been removed previously.

Nematocides Used

Allyl alcohol -- $C_3H_5OH$
A-A + D-D -- allyl alcohol + D-D
Bedrench -- allyl alcohol 81% + EDB 11.5%
C-1000 -- an isomeric mixture of compounds
CC-9882 -- diphenyl sulfide
Chloropicrin -- trichloro nitromethane
DBCP (Nemagon and Fumazone) -- 1,2-dibromo-3-chloropropane
D-D -- 1,3-dichloropropene-1,2-dichloropropane
Dorlone -- a mixture of EDB (18.7%) + Telone (75.2%)
EDB -- ethylene dibromide
Cynem (EN-18133) -- O,O-diethyl O-2-pyrazinyl phosphorothioate
EN-28450 -- 2-allylthio-2-thiozoline
EP-162 (Vorlex) -- methyl isothiocyanate + 1,3-dichloropropene
Formaldehyde -- HCHO (40%)
MC-2 -- methyl bromide 98% + chloropicrin 2%
Mylone -- 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione
N-dure -- urea formaldehyde
SD-4741 -- O,O,O-trimethyl phosphorothioate $(CH_3O)$ PS
Telone -- 1,3-dichloropropene (Tech.)
Phorate (Thimet) -- O,O-diethyl S-(ethylthio)methylphosphorodithioate
Vapam -- sodium N-methyl dithiocarbamate dihydrate

## RESULTS AND DISCUSSION

Obviously the application rate becomes a critical factor in an experiment where an effort is being made to determine the comparative effectiveness of a number of chemical compounds in the control of root knot. The use of slightly more than the optimum needed for control may make one compound appear very good, whereas less than the optimum of another may make another compound seem comparatively ineffective. Undoubtedly, since the exact quantity that represented the optimum for root-knot control in muck soil was not known in every instance and thus possibly was not used, some of the treatments cannot be properly ranked as to their effectiveness in nematode control. They can only be judged on the basis of the application rates as they were chosen for this experiment.

EDB, used at the rate of 15 gallons/acre, gave the most complete control of root knot of any of the treatments included in the experiment. The second choice is difficult, but possibly should be methyl bromide (MC-2), although both Fumazone and Cynem (EN-18133) also were very good. However, the effectiveness of the last two -- as well as of Nemagon -- apparently is regulated by the application method, since they were found to be comparatively ineffective against M. hapla when applied at even higher rates as sidedressings to rows of carrots and celery growing in muck soil (4), indicating that they do not move any appreciable lateral or vertical distance from the point of application in muck soil.

The selection of fourth place is also difficult since there was little to choose between the performance of Vapam, Mylone, EP-162, Nemagon, Bedrench (a mixture of EDB and allyl alcohol), and EN-28450 at the rates used. A further examination of the data on root-knot control would seem to place Telone in tenth place and D-D in eleventh. Dorlone, a mixture of EDB and Telone, did less well at the rate used than did Bedrench. The exact ranking of such materials as CC-9882, chloropicrin, formaldehyde, allyl alcohol, and phorate (Thimet) is virtually impossible to judge on the basis of the results obtained in this test. The poor performance of chloropicrin, when only a water seal was used to retard its rate of escape from the

soil, is rather difficult to understand, but it did give much better control when confined with the soil by a polyethylene tarp. N-dure, A-A + D-D, and C-1000 should have been used at a higher rate to obtain a degree of control comparable to the other materials used. SD-4741, which is rated as a soil fungicide rather than as a nematocide, showed the highest infestation of the experiment.

It is interesting to note that four treatments showed a higher infestation of root knot than that which represents an average of the two untreated check plots. This has happened before in several experiments of this kind (2, 3), where various treatments have given a similar result. In some instances there has been strong evidence that certain compounds have stimulated the hatching of M. hapla larvae, with a resulting increase in the amount of root-knot infestation present on the first planting of a host plant following the application of the chemical to the soil.

The average values relative to the degree of root-knot infestation (last column of Table 1) indicate that using a tarp to confine the chemicals in contact with the soil was more effective than a water seal, since there were more plants with readings of 0 and 1 and fewer with ratings of 2, 3, and 4 in the compartments where the soil had been tarped than where only a water seal was used. Also, of mathematical necessity, the average score for the total of 120 plants was considerably lower for the tarped samples.

In 10 of the 12 instances where tarping was compared with a water seal, the use of the polyethylene cover gave better nematode control than did simply wetting the surface of the soil with water. However, Cynem was an exception, and in the two instances in which this compound was used the control was actually poorer with tarping than with a water seal.

In a comparison of plant growth in tarped and untarped samples of soil (Table 2), the tomato vines averaged 11% larger in the tarped soils, the root systems were 32% heavier, and the fruit load was about 15% greater. However, even the tarped plots failed to equal the average growth in the untreated checks in any one of these three categories.

Table 2. Comparative plant growth (vines, fruits, and roots) in lots of treated soil when tarped and not tarped, in those where root-knot infestation was heavy and/or light, and in the untreated check plots.

| | Average weight in grams of: | | |
|---|---|---|---|
| Averages of various treatment groupings | vines | fruits | roots |
| Twelve lots of soil covered with polyethylene tarp | 147 | 63 | 12.3 |
| Twelve lots of soil not covered with polyethylene tarp | 132 | 54 | 11.3 |
| Twelve treatments permitting heavy infestation | 126 | 51 | 20.2 |
| Twelve treatments permitting only light infestation | 135 | 60 | 13.0 |
| Two untreated lots of soil (checks) | 150 | 75 | 15.7 |

Also, in a selection of 12 bench compartments that showed both low and high root-knot infestation (Table 2), the vine growth was 21% greater with low than with high root-knot damage, and the percentage difference in fruit yield was approximately the same as that for vine growth. However, the root systems actually weighed more where root knot was severe, possibly due to the fact that branching was more excessive, which in turn resulted in the development of many more fine roots.

The very wide spread in the degree of infestation between the 12 "best" and "poorest" treatments is indicated by the fact that none of the plants in the poorer treatments were entirely free of root knot, whereas only 29 plants of 120 from the better ones were found to show any knots at all.

The soil used in this experiment, which was obtained from a carrot plot in a crop rotation series, showed a considerable population of Pratylenchus spp. and a lesser one of Paratylenchus spp. in late October. However, specimens of these genera were recovered from only 11 and 3 of the 42 differently treated lots of soil, respectively, when the tomato plants were removed at the end of February. Nor were larvae of M. hapla very numerous in most of the 40 lots of soil as late as 2 months after the tomato plants were set.

The effect of confining the treatments with the soil by means of a polyethylene tarp was

even more striking in the data relative to the comparative numbers of larvae of M. hapla than were the differences in the degree of root-knot infestation, since the average number was at least five times as great in the untarped as in the tarped lots of soil. Again the failure of chloropicrin, allyl alcohol, phorate, and formaldehyde to control the root-knot nematode in the untarped samples was evident, since the larvae were nearly as numerous in those plots as in the untreated checks. That heavy root-knot infestation was accompanied by high larval counts for M. hapla, and conversely that there were few larvae present when root knot was low or absent, is well illustrated by the average counts for 12 different treatments in each grouping; namely, 5989 larvae versus 17 per pint of soil.

Another feature of the data on nematode populations in the differently treated lots of soil is the fact that the average number of free-living forms was larger in those samples where the larval counts of the plant-parasitic species were fewest in number, and vice versa. This suggests the possibility that some form of competition may exist between the free-living forms and M. hapla, or that some of the former are not so sensitive to the lethal effect of the nematocides used in this experiment as is M. hapla.

The leaves of the tomato plants growing in different lots of soil that had been treated with Cynem showed a very definite purplish mottling, and the roots of those plants growing in soil treated with DBCP (both Nemagon and Fumazone) showed a yellowish-brown surface discoloration. This latter effect has been observed on the roots of both tomato and lettuce in numerous other experiments in which this chemical has been used as a soil treatment (2, 3, 4).

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# A DELAYED HARVEST TEST OF FUNGICIDE SPRAYS ON TOMATOES RESISTANT AND SUSCEPTIBLE TO FRUIT CRACKING

R. W. Samson[1]

## Summary

The Improved Garden State, Tecumseh (7) and Roma (4) tomato varieties were subjected to 2, 4, and 6 weeks' delay of initial harvest in a test of fungicidal control of ripe fruit rots. Minute to conspicuous growth cracks and other epidermal breaks developed in fruit of the first two varieties under conditions of defoliation and fluctuating moisture. Fruit of Roma appeared quite resistant to such cracking. The fungicidal treatments effectively controlled early blight, gray leafspot and anthracnose fruit rot, caused by Alternaria solani, Stemphylium solani and Colletotrichum atramentarium, respectively. Sunburn of fruits was largely prevented through control of the leaf blights. The treatments apparently were ineffective in control of decays caused by crack-invading pathogens, of which A. tenuis may have been predominant. Crack rots destroyed most ripe fruit of the first two varieties where harvest was delayed beyond a 2-week period, thus nullifying the control of anthracnose fruit rot and prevention of sunburn achieved with the fungicide treatments. In marked contrast, ripened fruit of fungicide-sprayed Roma accumulated and persisted in decay-free condition through a 6-week delay of harvest.

## INTRODUCTION

Harvesting of tomatoes grown for processing is usually delayed until sufficient ripe fruit has accumulated to justify a harvesting operation. Successful use of mechanical harvesters now in experimental stages of development may require rather extreme delays, since the bearing plants must be sacrificed in the harvesting operation. Such delays invite loss from fruit rots, particularly in the midwestern and eastern United States where rains may fall on varieties whose fruits are susceptible to varied forms of epidermal cracking. Such loss may be partly avoided by weekly or more frequent picking of ripened fruit, before development of decays. But such frequent harvesting of processing tomatoes is seldom feasible. Harvest delays, or intervals between repetitive harvests, often may be prolonged. As a consequence substantial losses from fruit rots occur in rainy or humid seasons, despite fungicide applications.

Some of the rots are caused by pathogens capable of direct penetration of uninjured fruits. Colletotrichum atramentarium (2), the cause of anthracnose fruit rot, is the prime example in the humid midwest and east. It is of major concern to the tomato processing industry and prodigious efforts are made to control it with fungicides.

Other decays may be caused by microorganisms gaining entrance into fruits only through growth cracks, skin checks, and sunburns. These epidermal breaks commonly occur in most varieties grown for processing. The widespread and weakly pathogenic Alternaria tenuis most often invades these breaks (1, 3, 9). The resulting Alternaria rot appears responsible for much destruction of fruit protected from anthracnose rot by fungicide applications.

Control of these two classes of tomato fruit rots was investigated at Lafayette, Indiana in 1960. Two types of varieties, a zero and two rates of fungicide application, and delays of 2, 4 and 6 weeks in initial harvest were used.

## EXPERIMENTAL PROCEDURE

Southern-grown transplants of the Improved Garden State, Tecumseh and Roma varieties were set in the field on May 21 to provide three replications of variety whole plots, planted at the rate of 4840 plants per acre. Each whole plot was split into three plots receiving 0, 3 and 6 pounds of manganese ethylene bisdithiocarbamate (maneb) fungicide per acre, respectively. These plots were each further split into three subplots assigned, respectively, to 2, 4 and 6 weeks' delay of initial harvest.

The Improved Garden State and Tecumseh varieties were chosen as representative round-fruited types generally grown for processing. Their several-loculed fruit are subject to epidermal cracking under fluctuating moisture or exposure to intense sun. Tecumseh possesses resistance to gray leafspot. Roma (4, 8) was chosen for its high yield and apparent resistance to fruit cracking. It produces two or three-loculed oblong fruit of medium size, commonly termed Italian pear or paste type.

Table 1. Yields of Improved Garden State subjected to zero and two rates of fungicide application and 2, 4 and 6 weeks' delay of initial harvest.

| Fungicide treatment and harvest dates | Yield in tons/acre after initial harvest delay of: 2 weeks | 4 weeks | 6 weeks |
|---|---|---|---|
| No fungicide | | | |
| Aug. 24 | 3.4 | -- | -- |
| Sept. 7 | 1.2 | 1.3 | -- |
| Sept. 21 | 0.0 | 0.0 | 0.0 |
| Totals | 4.6 | 1.3 | 0.0 |
| Maneb, 3 lb/acre | | | |
| Aug. 24 | 6.8 | -- | -- |
| Sept. 7 | 6.5 | 6.8 | -- |
| Sept. 21 | 4.8 | 4.2 | 5.2 |
| Totals | 18.1 | 11.0 | 5.2 |
| Maneb, 6 lb/acre | | | |
| Aug. 24 | 4.2 | -- | -- |
| Sept. 7 | 7.0 | 8.0 | -- |
| Sept. 21 | 6.2 | 5.9 | 8.4 |
| Totals | 17.4 | 13.9 | 8.4 |

Table 2. Yields of Roma subjected to zero and two rates of fungicide application and 2, 4 and 6 weeks' delay of initial harvest.

| Fungicide treatment and harvest dates | Yield in tons/acre after initial harvest delay of: 2 weeks | 4 weeks | 6 weeks |
|---|---|---|---|
| No fungicide | | | |
| Aug. 24 | 4.5 | -- | -- |
| Sept. 7 | 0.7 | 1.5 | -- |
| Sept. 21 | 0.0 | 0.0 | 0.0 |
| Totals | 5.2 | 1.5 | 0.0 |
| Maneb, 3 lb/acre | | | |
| Aug. 24 | 4.8 | -- | -- |
| Sept. 7 | 7.7 | 13.0 | -- |
| Sept. 21 | 9.6 | 10.8 | 23.7 |
| Totals | 22.1 | 23.8 | 23.7 |
| Maneb, 6 lb/acre | | | |
| Aug. 24 | 4.5 | -- | -- |
| Sept. 7 | 12.2 | 15.6 | -- |
| Sept. 21 | 10.6 | 11.9 | 25.7 |
| Totals | 27.3 | 27.5 | 25.7 |

The fungicide was applied weekly from July 21 to September 6. The three respective delays of initial harvest were calculated from the approximate first appearance of ripe fruit in the plantings. Two additional pickings were made from those plots assigned to a 2-week delay, one additional harvest was made from those assigned to 4 weeks' delay, but only the one final harvest was made from the 6 weeks series.

Only red-ripe fruit free of sunburn and visible decay were harvested and recorded. Improved Garden State and Tecumseh responded much the same to the treatments so the yields of the latter are not presented. The yields of Improved Garden State are shown in Table 1, arrayed according to harvest delays, fungicidal treatments and harvest dates. The Roma data are similarly presented in Table 2.

## DISEASE DEVELOPMENT AND CONTROL

Early blight and gray leafspot severely defoliated all non-fungicide plots of Improved Garden State and Roma. Unsprayed Tecumseh exhibited its resistance to gray leafspot but was defoliated by early blight. Defoliation resulted in extensive sunburn of fruit. Anthracnose developed abundantly in all non-fungicide plots, while crack rots destroyed much fruit of Improved Garden State and Tecumseh, irrespective of fungicide treatment.

Control of anthracnose, early blight and gray leafspot, and prevention of sunburn was good with either rate of fungicide. Yields indicated that disease control was about 14% better with

## RESULTS WITH IMPROVED GARDEN STATE

The low yields from non-fungicide plots of this variety (Table 1) were due to defoliation, sunburn, anthracnose and crack rots. Breakdown of plants and fruit left no marketable tomatoes at the end of the 6-week period. Where either rate of fungicide was applied, ripe fruit did not accumulate and persist in decay-free condition beyond a 2-week delay of harvest. Cracking of the fruit permitted invasion by *Alternaria tenuis* and other decay organisms.

## RESULTS WITH ROMA

As with Improved Garden State, breakdown of plants and fruit in non-fungicide plots of Roma (Table 2) prevented harvest of marketable tomatoes at the end of the 6-week period. This breakdown was due to defoliation and consequent sunburn, and to anthracnose fruit rot. A minimum of decay attributable to crack-invading organisms was noted. Where either rate of fungicide was applied, ripe fruit of Roma accumulated and persisted in decay-free condition through a 6-week delay of harvest. Where initial and only harvest was delayed 6 weeks the yield essentially equaled the total of two harvests from plots subjected to 4 weeks' delay of initial harvest or of three harvests from plots subjected to 2 weeks' initial delay.

## DISCUSSION

The Roma variety has considerable current utility for processing as whole tomatoes or products. Single harvests of 25 to 30 tons per acre were taken from numerous Indiana Roma fields in 1960. Its fruit cracking resistance may be of greater interest. Such resistance, evident through virtual elimination of crack rots, obviously enhanced returns from fungicide applications. In a fungicide evaluation trial in 1960 Roma proved more useful than two other varieties in disclosing differences in effectiveness of various formulations in control of anthracnose and the foliage diseases because crack rots were not obscuring factors. Possibly Roma can be used in tomato fungicide evaluations without resorting to laborious repetitive harvests.

It is not clear whether the apparent resistance to crack rots of the Roma variety can be attributed to the same kind of fruit cracking resistance of certain varieties of the roundfruited type recently released by plant breeders (5, 6, 8). Apparently the resistance of these new varieties may provide a direct control of crack rots and enhance the return from fungicides used to control pathogens capable of direct penetration of fruits.

A varied scheme of delayed harvesting plus fungicide treatment might provide a useful test of resistance to fruit cracking if conducted under rainy or humid conditions favoring both the cracking and infection by various diseases of fruit and foliage.

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# OTHER LEGUMES PROVE SUSCEPTIBLE TO A POWDERY MILDEW OF PSORALEA TENAX[1]

Howard W. Johnson and John P. Jones[2]

When plants of Psoralea tenax, P. glandulosa, and P. bituminosa were grown on a greenhouse bench at Stoneville, Mississippi, the leaves of P. tenax became heavily coated with powdery mildew mycelium and spores, while those of the other two species remained mildew-free (Fig. 1A). The leaf blades and petioles of Dorman soybean plants (Glycine max) growing on a nearby bench also developed powdery mildew lesions (Fig. 1B). Apparently the mildew had spread from P. tenax to Dorman soybean by natural means. A test was conducted, therefore, to determine whether the mildew could be spread from P. tenax to other leguminous species by artificial inoculation.

To make the inoculation, several heavily mildewed plants of P. tenax were placed in front of the air inlet inside an inoculation chamber. A fan was then started outside the chamber and the mildew conidia were blown over the pots of plants to be inoculated, which had previously been placed near the other end of the inoculation chamber. After 48 hours' exposure to the spore shower, the plants were placed on a greenhouse bench and observed regularly for mildew development.

Eight pots of Korean lespedeza plants (Lespedeza stipulacea) and four pots of plants of each of the following six varieties of soybean were inoculated: Arksoy, Dorman, Illini, Mukden, Ogden, and Roanoke. Mildew symptoms were evident on plants of Korean lespedeza in each of the eight pots within 5 days. Mildew colonies were present also on the leaf blades, petioles and stems of all 20 plants of the following varieties of soybean within 5 days: Arksoy, Dorman, Ogden, and Roanoke. More mildew developed on Dorman than on the other three soybean varieties, but all four varieties were susceptible. The plants of Illini and Mukden soybeans remained mildew-free and these varieties appeared to be highly resistant to, or immune from, the strain of mildew. Two pots of Korean lespedeza and one pot of each soybean variety were left in another greenhouse section under comparable conditions to serve as non-inoculated checks. These plants all remained free from mildew.

Three pots of plants of each of the following varieties of lupine placed on a greenhouse bench adjacent to the inoculated plants of Korean lespedeza also developed powdery mildew: 1) Imported yellow lupine, F.C. 33,163[3] (Lupinus luteus), 2) Borre sweet blue lupine, F.C. 33,506 (L. angustifolius), and 3) Hasting's white lupine, F.C. 33,866 (L. albus). The mildew covered completely the leaves of yellow and blue lupines, which appeared highly susceptible. It occurred only as discrete colonies on the leaves of Hasting's white lupine. This variety seemed, therefore, to possess slight or moderate resistance to the strain of mildew.

Mildew-infected plants of P. tenax, soybean, lespedeza, and lupine were kept in the greenhouse until maturity and were observed regularly for perithecia of the causal fungus but none were found. Nor did perithecia form on mildew-infected plants of P. tenax transplanted from the greenhouse to a field nursery row and grown to maturity. In the absence of the perithecial stage, the powdery mildew species was not identified with certainty. The Index of Plant Diseases in the United States (4) lists two species of powdery mildew on Psoralea: Erysiphe polygoni DC. on P. tenuiflora and Microsphaera diffusa Cke. & Pk. on P. physodes. The latter species of powdery mildew was identified from its perithecial stage on Korean lespedeza by Johnson, Lefebvre, and Ayers (1); on soybean by Lehman (2), and on blue lupine by Thompson (3). It seems probable, therefore, that the powdery mildew species involved in these studies is M. diffusa. This is the first report of powdery mildew on P. tenax in the United States.

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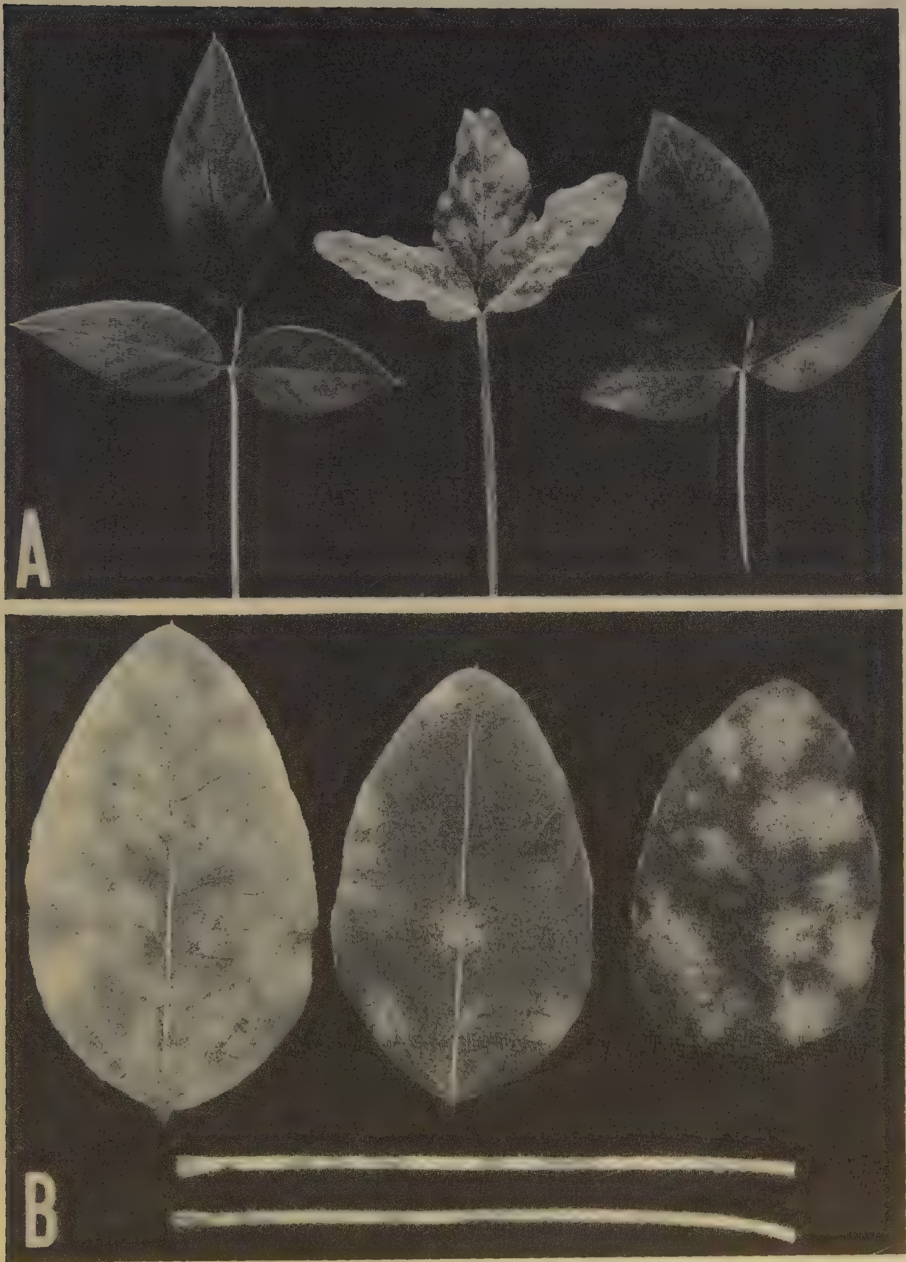


FIGURE 1.
A -- Mildewed leaf of Psoralea tenax (center) compared with powdery mildew-free leaf of P. glandulosa (right) and of P. bituminosa (left) from plants of the three species grown on the same greenhouse bench.
B -- Mildewed leaf blades and petioles of Dorman soybean plants infected by the powdery mildew from P. tenax.

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# STORAGE OF FUNGICIDE-TREATED PEA AND CUCUMBER SEEDS[1]

D. J. deZeeuw, R. A. Davis and R. A. Crum[2]

Abstract

Pea and cucumber seeds treated with fungicides were stored up to 11 months in sealed jars at room temperature and in the refrigerator. No obvious reduction in germination or vigor of either type of seed resulted from storage. Peas appeared to retain more viability when stored at the lower temperature but differences were small. No benefit from low temperature storage was found for the cucumber seed. In this work there was no evidence of increased injury with time of storage of seeds treated with either volatile (Panogen 15) or non-volatile (Elcide 73) mercury seed treatment. The presumed damage to peas by volatile mercury occurred soon after treatment. The non-volatile mercury material gave significantly improved emergence and stand comparable to that provided by thiram (Arasan 75). Cucumber seed tolerated both types of mercury seed treatment equally as well as thiram and protection against damping off was retained for the full storage period at either temperature. All the treatments protected cucumber seedlings against post-emergence root damage as shown by green weight of seedlings.

Certain of the more volatile mercury seed treatment fungicides have been reported toxic to various seeds (3, 5, 7). The effects are most pronounced on seeds sensitive to a specific material, as is the case with peas, beans and limas. Other seeds, generally not harmed, may be injured by prolonged storage especially in an unfavorable environment. Excessive seed moisture, mold growth, seed coat injuries, and unfavorable temperatures have been variously reported as contributing to injuries produced by fungicides (2, 6, 8, 9, 10, 12, 13). Generally low temperatures are considered favorable for seed storage (11), but on occasion low temperature or fluctuating temperatures in open sheds have been responsible for injury with mercury compounds (9). Seed moisture presumably fluctuates also under these conditions.

Since it is possible for the volatile materials to dissipate prematurely in common storage (1) or to become more toxic in a closed environment, some of the storage effects of both volatile and non-volatile materials were tested on pea and cucumber seeds.

## MATERIALS AND METHODS

Seeds of Alderman peas[3] and SMR-12 pickling cucumber were weighed out in lots of 580 grams and 60 grams respectively. Half of the seed lots were numbered 1, 3, 5, 7, 9 and reserved for storage in sealed mason jars at room temperature and the other half -- even numbered 2, 4, 6, 8, and 10 -- were held in the same type containers in an ordinary refrigerator. Treatments with the fungicides were applied by the slurry or wet method and the seeds were dried for 1 hour before sealing in the storage containers (Table 1). Controls were treated with a small amount of water equivalent to that applied with fungicide slurries. At the various planting dates (0, 5, 7, 9, and 11 months) six replicate seed lots of 60 seeds from each storage unit were counted and planted at uniform depth in greenhouse flats containing a mixture of unsterilized muck and mineral soil. After emergence damped off seedlings were counted, removed and summed for the experimental period. At the conclusion of each growing period the surviving seedlings were pulled, counted, and observed for disease and general vigor. In several cases seedlings were cut at the ground line and green weight obtained. Data were analyzed statistically by the standard analysis of variance, where results appeared to warrant further analysis.

Treatments were applied to the seeds and storage was begun April 27, 1960. The first planting (0 months) was made on April 28. Thereafter plantings were made on Sept. 16 (5

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[3]Seeds supplied by Ferry-Morse Seed Co.

Table 1. Alderman peas and SMR-12 pickling cucumber seed: storage condition, fungicide treatment materials and rates used.

| Treatment number | Storage condition | Material | Rate (ounces/100 pounds of seed) peas | cucumber |
|---|---|---|---|---|
| 1 | room | Elcide 73[a] | 3/8 | 3/4 |
| 2 | refrig. | Elcide 73 | 3/8 | 3/4 |
| 3 | room | Elcide 73 | 3/4 | 1 1/2 |
| 4 | refrig. | Elcide 73 | 3/4 | 1 1/2 |
| 5 | room | Panogen 15[b] | 3/8 | 3/4 |
| 6 | refrig. | Panogen 15 | 3/8 | 3/4 |
| 7 | room | Arasan 75[c] | 2 | 3 |
| 8 | refrig. | Arasan 75 | 2 | 3 |
| 9 | room | Control | -- | -- |
| 10 | refrig. | Control | -- | -- |

[a]12% aqueous sodium ethylmercurithiosalicylate. Eli Lilly Co. "Merthiolate"
[b]2.2% methyl mercury dicyandiamide. Morton Chemical Co.
[c]tetramethylthiuram disulfide (thiram 75% wettable powder.) E. I. duPont deNemours & Co.

Table 2. Storage of fungicide-treated Alderman pea seed. Total emergence, final stand, and root rot ratings 2 weeks after planting in unsterilized mineral soil and muck mixture.

| Treatment number | Total average emergence[a] at ___ months' storage: 0 | 5 | 7 | 9 | 11 | Final average stand at ___ months' storage: 0 | 5 | 7 | 9 | 11 | Root rot range[b] at 5 months |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8.8 | 11.7 | 10.0 | 4.5 | 4.5 | 4.2 | 6.0 | 4.7 | 2.0 | 1.7 | 1.75 |
| 2 | --- | 21.2 | 24.3 | 14.0 | 16.1 | --- | 11.5 | 12.8 | 6.2 | 9.3 | 1.50 |
| 3 | 21.2 | 28.3 | 27.0 | 13.0 | 21.7 | 14.0 | 20.0 | 16.5 | 7.0 | 13.3 | 1.50 |
| 4 | --- | 37.5 | 35.8 | 25.7 | 30.1 | --- | 26.2 | 25.7 | 16.5 | 19.1 | 1.58 |
| 5 | 1.5 | 3.8 | 1.2 | 2.5 | 1.1 | 1.0 | 2.3 | 0.7 | 0.8 | 0.8 | 1.90 |
| 6 | --- | 5.3 | 2.8 | 5.3 | 2.8 | --- | 3.0 | 1.5 | 0.7 | 1.3 | 1.65 |
| 7 | 16.8 | 23.8 | 15.8 | 13.0 | 14.7 | 11.5 | 10.7 | 8.7 | 4.5 | 7.8 | 2.04 |
| 8 | --- | 17.3 | 21.3 | 17.0 | 20.1 | --- | 9.3 | 9.3 | 7.2 | 9.0 | 2.12 |
| 9 | 4.3 | 2.3 | 2.3 | 1.7 | 1.1 | 3.5 | 1.3 | 0.8 | 0.5 | 0.7 | 2.10 |
| 10 | --- | 4.3 | 2.5 | 1.0 | 2.0 | --- | 2.5 | 1.0 | 0.3 | 0.7 | 1.80 |
| L.S.D. 1% | | 7.5 | | | 6.4 | | 6.6 | | | 5.1 | |

[a]60 seeds per plot.
[b]1--some lesioning and root browning; 2--extensive lesioning and root browning; 3--very severe but top still not wilted; 4--dead plants as a result of root rot. Average of all surviving plants in the treatment.

months), Nov. 10 (7 months), Jan. 13, 1961 (9 months), and Mar. 15, 1961 (11 months).

## EXPERIMENTAL RESULTS

Peas: Emergence, stand and root rot data for peas are summarized in Table 2. The lower storage temperature appeared to be beneficial to pea emergence and stand in this experiment. Differences were small and often obscured by the very low stands but were nevertheless fairly consistent. Germinative capacity at either storage temperature was nearly the same at 11 months as in the shorter storage periods. No clear evidence of chemical injury aggravated by prolonged storage can be demonstrated at either temperature. While seed injury may have resulted from treatment with the volatile mercury material, Panogen 15, it probably occurred before the storage period was begun (note the data for cucumber).

The results on Elcide 73 confirm previous field experiments (4) in which Elcide 73 was found to be relatively non-injurious to peas and beans and equal in effectiveness to some established standard non-metallic materials for damping off control.

With Elcide treated seed, root-rot and browning was less severe on the average than that in the controls or other treatments. The degree of root browning, however, was quite variable and no definite conclusions can be drawn from these data.

Cucumbers: The cucumber situation was significantly different from peas in several respects. The emergence and stand figures show that neither form of mercury seed dressing was injurious (Table 3). All of the materials produced excellent stands of vigorous plants compared with no treatment. Both pre-emergence and post-emergence damping off were severe and test conditions were such that ineffective or toxic materials would have been evident. Time of storage was not a factor in reduction of stand or germination potential within the length of the experiment.

Table 3. Storage of fungicide-treated SMR-12 cucumber seeds. Total emergence, final stand and average weights of plants at 5 months' and 11 months' storage.

| Treatment number | Total average emergence[a] at ___ months' storage: 0 | 5 | 7 | 9 | 11 | Final average stand at ___ months' storage: 0 | 5 | 7 | 9 | 11 | Average weight/plant (g): 5 months | 11 months |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 52.0 | 58.5 | 57.7 | 55.8 | 57.7 | 44.7 | 56.8 | 50.7 | 49.2 | 51.0 | 1.16 | 1.35** |
| 2 | ---- | 58.3 | 58.8 | 57.8 | 58.0 | ---- | 55.5 | 51.8 | 51.2 | 53.3 | 1.15 | 1.39** |
| 3 | 58.3 | 59.2 | 58.7 | 57.3 | 57.5 | 55.7 | 58.5 | 56.3 | 50.8 | 49.5 | 1.16 | 1.34** |
| 4 | ---- | 60.0 | 58.5 | 57.5 | 59.1 | ---- | 58.2 | 53.3 | 50.2 | 54.8 | 1.14 | 1.33* |
| 5 | 56.3 | 59.8 | 58.5 | 56.8 | 58.7 | 51.5 | 58.0 | 55.5 | 50.2 | 54.0 | 1.11 | 1.31* |
| 6 | ---- | 59.5 | 58.7 | 57.3 | 58.5 | ---- | 57.5 | 50.5 | 49.2 | 53.5 | 1.13 | 1.34** |
| 7 | 59.0 | 58.8 | 58.5 | 57.7 | 58.7 | 52.3 | 57.5 | 54.5 | 49.7 | 54.0 | 1.13 | 1.35** |
| 8 | ---- | 58.0 | 57.2 | 57.8 | 59.0 | ---- | 56.3 | 53.2 | 48.0 | 51.3 | 1.10 | 1.37** |
| 9 | 24.7 | 47.3 | 31.8 | 30.2 | 40.7 | 18.5 | 38.0 | 19.2 | 18.3 | 21.0 | 1.01 | 1.14 |
| 10 | ---- | 46.0 | 31.0 | 32.5 | 42.5 | ---- | 31.7 | 17.3 | 17.3 | 23.8 | 0.99 | 1.17 |
| L.S.D. 5% | | | | | 4.0 | | | | | 6.5 | n.s. | 0.13 |
| L.S.D. 1% | | | | | 5.3 | | | | | 8.7 | n.s. | 0.17 |

[a]60 seeds per plot.
[b]* Superior to either control at 5% level of significance; ** at the 1% level.

Storage temperature had little or no effect on the cucumber seed germination or seedling stand either at 5 months' or after the full 11 months' storage. There was no consistent temperature effect associated with treatment or lack of treatment in any case. Cucumber seeds have retained relatively high viability (above 90%) in common dry storage in this laboratory for 2 years, so little increase in germination can be expected from refrigeration.

Resistance of treated seeds to damping off was obvious in both stand and emergence figures. Control plants, even with less competition for space, were smaller and more deformed than any from treated seed. The relative size was visually correlated with root vigor and in all cases seed treatment was apparently responsible for a much fuller and cleaner root system.

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# CULTURAL TYPE OF VERTICILLIUM ALBO-ATRUM IN NEW MEXICO[1]

T. E. Smith[2]

Verticillium albo-atrum Reinke & Berth. of three cultural types (1, 2) occurs on Irish potatoes: white, dark mycelial, and microsclerotial. Cultural type was of significance in the investigations cited because the microsclerotial type was difficult to control by crop rotation while the dark mycelial type was controlled by 2 years of resistant crops. Cotton was susceptible to both the dark mycelial and microsclerotial types (2). This report summarizes the results of an examination of cultural type of Verticillium in New Mexico.

The host plants from which Verticillium was isolated included cotton, okra, peanut, sesame, Russian olive, chilli pepper, tomato, eggplant, mint, castorbean, sweetpotato, and several species of weeds. The cotton was collected from the major cotton-growing counties of New Mexico, and the other plants came mainly from a host plant nursery on naturally infested soil at the Agronomy Farm of the New Mexico Agricultural Experiment Station.

Chips of discolored stele were planted on potato-dextrose-carrot agar with 40 ppm of streptomycin nitrate. Isolations and subsequent culturing was done at 28°C. After verification of Verticillium by examination of conidiophores on the agar plates used for isolation, cultures were established by transfer to agar slants for cultural type examination, which was made by microscopic examination.

In all, 111 isolates were examined over a 3-year period. Some isolates produced microsclerotia in small numbers and after growth for a month. Others produced microsclerotia in abundance in a week, giving a tar black appearance. Nevertheless, all isolates were of the microsclerotial type. It appears that the microsclerotial type is the principal cultural type of Verticillium in New Mexico, and that the dark mycelial and white types are absent or rare in occurrence.

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# REACTION OF ALFALFA TO VERTICILLIUM ALBO-ATRUM[1]

T. E. Smith[2]

Alfalfa is attacked by *Verticillium* in Europe (1, 2), but apparently the disease is not known or at least is very uncommon on this crop in the United States. Alfalfa is the principal rotation crop grown on cotton land in certain areas of New Mexico, and its possible susceptibility to *Verticillium* is of considerable importance. Credence is given the susceptibility of alfalfa by successful inoculation of the Ranger variety by German investigators (2). Parker (3) summarized the literature and pointed out that there was no record of inoculation of alfalfa with cultures from other hosts.

To investigate this question, alfalfa was inoculated in the greenhouse and grown on naturally infested soil in the field, using the Ranger, New Mexico 11-1, New Mexico Common, Lahontan, Zia, and African varieties.

Inoculations were made with five cultures of the microsclerotial type, isolated from cotton, by dipping the roots of these varieties in macerated cultures prior to transplanting into soil. The inoculated plants were kept at a temperature favorable to infection, approximately 70° F. Cotton inoculated with these cultures became diseased in about 2 weeks. After 4 to 12 weeks' incubation, a few of the alfalfa plants developed yellowing, but mainly the plants were similar to the uninoculated controls. Isolation of *Verticillium* was attempted by planting chips of stele from the inoculated alfalfa, 4 or more weeks after inoculation, on potato-dextrose-carrot agar with streptomycin. In all, isolations were attempted from 109 alfalfa plants. Only seven of these gave *Verticillium* which was of the microsclerotial type and similar to the cultures used for inoculation.

The same varieties were grown in naturally infested soil in the field by transplanting alfalfa seedlings into the cotton rows and thinning the cotton to one plant 12 inches from each alfalfa plant. Approximately half of the alfalfa plants were a year old at the time of transplanting, having been grown in pots inoculated with *Verticillium*. All of the cotton plants became diseased from *Verticillium*. Isolations were attempted from the root crown of 172 mature alfalfa plants. No *Verticillium* was isolated, although *Rhizoctonia* and *Fusarium* occurred in some of the alfalfa roots.

Alfalfa was highly resistant to the *Verticillium* attacking cotton as shown by reisolation of this fungus from such a low percentage of the inoculated plants and the absence of *Verticillium* in alfalfa grown on naturally infested soil in the field. It appears that the *Verticillium* attacking cotton in New Mexico is a different biotype from that attacking alfalfa in Europe.

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# UNCOLLAPSED FIBERS ASSOCIATED WITH BOLL ROT IN COTTON

Paul B. Marsh and Thomas Kerr[1]

Cotton bolls exposed to wet weather at the time of opening frequently are severely attacked by microorganisms. The fiber becomes discolored, fails to fluff normally, and is often distinctly weakened. A boll may open partially or not at all. The symptom complex is commonly designated by the general term "boll rot." The term "microbial tight lock" has also been used to describe the somewhat less severe condition in which the fiber undergoes microbial damage but the boll opens sufficiently to allow the seed cotton to be harvested (4). Diplodia sp. (4), Alternaria (4, 6), Nigrospora (2), Aspergillus niger v. Tiegh. (7), and Aspergillus flavus Lk. ex Fr. (3, 5) have been associated with one or another type of boll rot.

In the present investigation fibers from bolls collected in the field in the microbial tight lock condition were examined under oil[2] and noted to exhibit the appearance shown in Figure 1. The fiber obviously had not collapsed in the normal fashion and the open lumen contained an air bubble. The fibers of Figure 1 were from bolls infected in the field in California with Nigrospora sp.[3]. Microbial tight locks from South Carolina infected with Diplodia contained numerous fibers with a very similar appearance, as did also tight lock fibers from Texas infected with Aspergillus flavus in a fluorescent-fiber condition described previously (3). It was apparent that the fibers showing the appearance seen in Figure 1 were not collapsed in the normal manner characteristic of undamaged fibers.

After the above observations had been made on field-infected fibers, experiments were carried out to determine whether fungi growing on cotton in pure culture could cause the uncollapsed fiber condition. Mature cotton bolls, almost ready to open, were obtained from the greenhouse, surface-sterilized with flaming alcohol, cracked open slightly by a blow with a small sterile hammer, and placed with sterile precautions into sterile quart fruit jars used as moist chambers in a manner previously described (3). Spores from a pure culture of a fungus were then dusted upon each boll and incubation carried out at approximately 30° C for 7 or 14 days. At the end of the incubation period the bolls were taken out of the jars, the seed cotton removed from them and dried overnight at 40° in a mechanical convection oven, and the fibers then observed under immersion oil. In most samples many uncollapsed fibers similar to those found in field-infected fibers were seen. Uncollapsed fibers were difficult to count accurately, especially because they tended to rise to the top of the mount. Any fiber containing a dark uncollapsed section more than five times the fiber's width was recorded as uncollapsed. Of eight fungi used in this experiment, seven caused the appearance of considerable numbers of uncollapsed fibers.

The percentages of uncollapsed fibers recorded in experiments with each of eight fungi were as follows: Alternaria #177, 46%; Aspergillus flavus #153, 28%; Aspergillus niger #145, 53%; Aspergillus terreus Thom #182, 50%; Fusarium moniliforme Sheldon #1004.1, 38%; Nigrospora sp., 70%; Penicillium #150, 56%; Rhizopus stolonifer (Ehr. ex Fr.) Lind. (R. nigricans Ehr.) ATCC 10404, 3%. No emphasis is laid upon the differences in percentages of uncollapsed fibers brought about by incubation with the above fungi except that the fibers incubated with Rhizopus stolonifer obviously gave a very low value. Check counts made on high grade unweathered cotton of the variety Coker 100 grown at 13 different locations across the cotton belt from North Carolina to California in 1948 yielded percentages of uncollapsed fibers ranging from 0 to 6. Five high grade cottons of widely diverse genetic background also were examined and all exhibited low percentages of uncollapsed fibers as follows: Garo Hill, 0%; Amsak, 6%; Tanguis, 0%; Pima S-1, 2%; Lengupa, 4%.

Uncollapsed fibers in commercial cotton were noted earlier by Bailey (1), who referred to them as "doughnut fibers" because of their appearance in cross-section and believed them to be associated with difficulties in spinning, particularly with excessive amounts of yarn breakage during the spinning process. Since microbial action is not known to be the only cause of uncollapsed fibers, it cannot be stated with certainty that Bailey's "doughnut fibers" originated in a microbial tight lock situation, although the rather common occurrence of microbial tight locks in the field makes this seem a reasonable possibility.

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[2] Crown Immersion Oil. Use of a product name does not imply recommendation by the United States Department of Agriculture to the exclusion of other products which may also be suitable.

[3] Material kindly supplied by Dr. Richard Garber, Shafter, California.

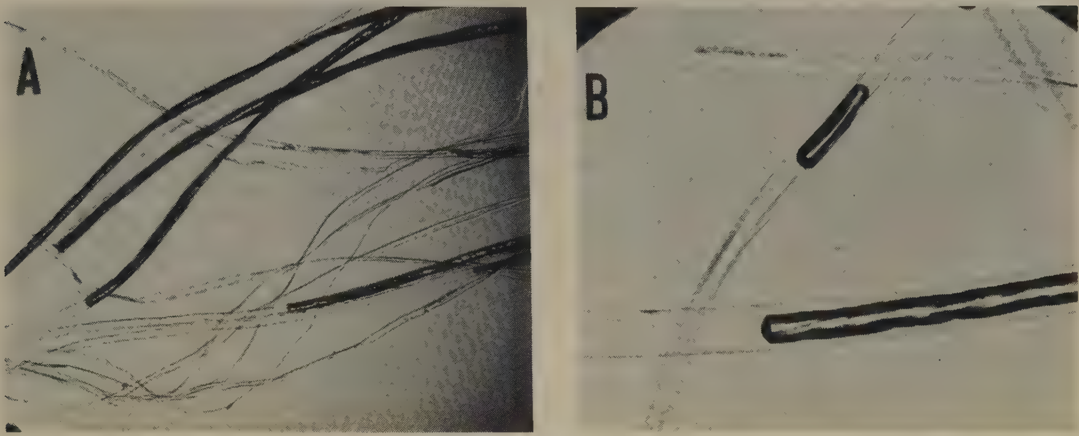

FIGURE 1. Microscopic appearance under oil of raw cotton fibers from a boll naturally infected in the field with Nigrospora. A -- fibers under low power; B -- fibers under high power.

Examination under oil is a simple method of detecting uncollapsed fibers. It is not claimed that precise quantitative counts can be made. However, if large differences in numbers of such fibers exist among samples under observation, the method may have value in carrying out fiber deterioration investigations with raw cotton.

The mechanism by which microbial attack brings about the occurrence of uncollapsed fibers is not known, but it seems highly probable that actual cellulosic degradation is involved. The one fungus of eight used in our pure culture tests detailed above which failed to cause uncollapsed fibers was Rhizopus stolonifer, a member of the Mucorales. Fungi of this order are generally regarded as being extremely low or entirely lacking in cellulose-decomposing ability (8). The process of fiber collapse and convolution involves the setting up of internal strains between cellulose chains within the fiber and apparently microbial decomposition renders the fiber basically incapable of going through this process.

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# SOIL AND FOLIAR TREATMENTS FOR THE CONTROL OF SCLEROTINIOSE OF LETTUCE[1]

J. F. Darby[2]

## Summary

Tests conducted from 1955 to 1961 on naturally infected mineral and peat soil, employing both chemical and cultural methods of control, suggested that a combination of each of the following control measures was necessary to obtain satisfactory control of sclerotiniose of lettuce: 1) flooding the soil for 6 weeks during the summer, or treating the soil with calcium cyanamide, 21% N, at the rate of 1000 pounds per acre 3 months before planting; 2) rotating the lettuce plantings with crops not so susceptible to the disease; 3) deep plowing to bury sclerotia; and 4) spraying 6 to 10 times at weekly intervals with one of the following: (a) ferbam, 76W-hydrated lime mixture (2.0 lb each per 100 gallons per acre per application); (b) PCNB, 75W-ferbam, 76W mixture (1.0 to 2.0 lb each); (c) thiram, 65W (2.0 lb); or (d) PCNB, 75W (2.0 lb).

Two applications using high dosages applied early in the growth of the crop were more phytotoxic and less effective than weekly applications using smaller dosages.

## INTRODUCTION

Sclerotiniose or lettuce drop, caused by Sclerotinia sclerotiorum (Lib.) d By., is widespread and commonly very destructive wherever lettuce is grown in Florida. Flooding the soil to rot the sclerotia, rotating with crops that are not attacked by the fungus, deep plowing to bury sclerotia, and treating the soil with calcium cyanamide have been recommended and used to control lettuce drop in Florida for a number of years, but the results have not been consistently satisfactory. Flooding is not economical for some farmers and calcium cyanamide has been phytotoxic on some soils. Practically all of the winter-grown crops, including celery, tomatoes, beans, cabbage, and eggplant, are susceptible to this disease in varying degrees of severity.

The present study, extending from 1955 to 1961, consists of a re-evaluation of the above treatments and combinations of them with soil and foliar applications of chemicals.

## METHODS

This paper is a report of 14 field experiments employing a total of 127 treatments. Most of the treatments were replicated four times, a few were replicated six times, and all plots were randomized within each block.

Plots on organic soil (Everglades mucky peat) were 6 feet wide and 57 feet long. Plots on mineral soil (Leon fine sand) were 5 feet wide and 57 feet long. Each plot on mineral soil consisted of three rows of lettuce planted with an inter-row spacing of 15 inches and inter-plant spacing of 12 inches. On organic soil each plot consisted of four rows with an inter-plant and inter-row spacing of 12 inches. To minimize drifting of materials, a treated, unplanted row was left between each plot.

Lettuce seedlings, variety Great Lakes, were grown in seedbeds treated with methyl bromide or an emulsible mixture of 1,3-dichloropropene-1,2-dichloropropane (D-D) and allyl alcohol and transplanted to naturally infested mineral soil. On peat soil, the lettuce was seeded directly in the field in some tests and transplanted in others.

Flooding was done from July 15 to August 25 when conditions were more favorable for rotting sclerotia (high temperature) and for providing water (heavy rainfall). Continuously flooded plots were 4 to 6 inches under water at all times. Intermittently flooded plots were under water during the day while at night the level was allowed to drop slightly below the soil surface. A part of the flooded area was treated with soil fumigants and fungicides after flooding (Table 1).

The intervals between the application of calcium cyanamide and transplanting were 1, 1 1/2, and 2 months. The total amount of nitrogen applied to each plot was equalized by adding low biuret urea to those plots lacking nitrogen in the treatment.

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Table 1. Effect of flooding and chemicals on yields of marketable lettuce. Data collected during winter of 1955-56 at Sanford, Florida.

| Flooding treatments | Chemical treatments[a] A | B | C | D | E | F | Total marketable heads |
|---|---|---|---|---|---|---|---|
| 1. Continuously flooded | 105 | 76 | 134 | 80 | 130 | 115 | 640 |
| 2. Intermittently flooded | 156 | 147 | 135 | 143 | 156 | 152 | 889 |
| 3. Not flooded | 24 | 13 | 29 | 13 | 43 | 27 | 149 |
| Totals | 285 | 236 | 298 | 236 | 329 | 294 | |
| LSD 5% | | | | | | | 78.9 |
| 1% | | | | | | | 109.1 |

[a]Treatment A consisted of PCNB, 75W, 30 pounds per acre; B, PCNB, 25E, 100 gallons; C, Stauffer's N-521 (Mylone), 90W, 167 pounds; D, Mylone, 85W, 235 pounds; E, allyl alcohol, 50 gallons; F, no chemical. Treatments A and B were applied as postplanting sprays; treatments C, D, and E were applied prior to planting. The reduction in yield in chemical treatments B and D, not flooded, was due mostly to phytotoxicity, while the reduction in the untreated F was due mostly to sclerotiniose.

Sodium N-methyldithiocarbamate (Vapam), dimethyl tetrahydrothiadiazine thione (Mylone), allyl alcohol, urea-formaldehyde concentrate, pentachloronitrobenzene (PCNB), ferric dimethyl dithiocarbamate (ferbam), 2,2'-methylenebis (3,4,6-trichlorophenyl) (hexachlorophene) (Nabac), and sodium dinitro-o-cresylate (Elgetol) were applied to the soil as drenches using approximately 10,000 gallons of water per acre. A tractor-drawn watering cart with mechanical agitation was used to apply the drenches. A minimum waiting period of 2 weeks was allowed between treatment and transplanting.

Half of one lettuce trial was planted in the same location for two seasons in succession while the other half was planted on an area planted to cabbage for two previous seasons.

Deep plowing to bury sclerotia was simulated by turning the top 8 to 10 inches of soil upside down with hand shovels.

Fungicides used for foliar and the surrounding-soil-surface treatments included PCNB, ferbam, PCNB-ferbam mixtures, ferbam-hydrated lime mixture, ferbam-sulfur mixture, ferbam-tetramethylthiuram disulfide (thiram)-PCNB-sulfur mixture, ferbam-streptomycin sulfate-2,4 dichloro-6-(o-chloroanilino)-s-triazine (Dyrene) mixture, Dyrene, thiram, urea-formaldehyde concentrate, ferbam-manganese ethylene bisdithiocarbamate (maneb) mixture, ferbam-zinc dimethyl dithiocarbamate (ziram) mixture, ferbam-ziram-PCNB mixture, disodium ethylene bisdithiocarbamate (nabam) plus zinc sulfate, hydrated lime, sulfur, tetrachloro-p-benzoquinone (chloranil), 2-pyridinethione 1-oxide (Omadine) zinc salt, Omadine ferric salt, hexachlorophene (Nabac), maneb, zinc ethylene bisdithiocarbamate (zineb), 2,3-dichloro-1,4-naphthoquinone (dichlone)-ferbam-sulfur mixture (Phybam-S), and Dyrene-streptomycin sulfate mixture. These treatments were applied with a tractor-mounted power sprayer equipped with mechanical agitation. The concentrations varied from 1/2 pound to 15 pounds per 100 gallons per acre and the number of applications varied from one to ten. When possible, applications were made just after cultivation in an effort to maintain a fungicide cover over the soil surface at all times. The spray was directed at the plants and the soil surrounding them at the rate of 100 gallons per acre per application. Weekly applications were begun just after the first cold weather or when the plants grew large enough to provide shade underneath. They were continued until 2 weeks before harvest, making a total of 6 to 10 applications. When only two applications were used, the first one was made 1 week after the plants were set in the field or 5 weeks after seeding directly in the field, and the other was made 2 weeks later. To minimize spray residue, the last application was made from 1 month to 2 weeks before harvest. Detailed information on all of the unsatisfactory treatments is not included in this paper.

## DISCUSSION OF RESULTS

Flooding: The percentage of heads showing symptoms of sclerotiniose in the flooded plots was 11.5 and in the non-flooded plots was 24.0. The number of marketable heads in intermittently flooded plots was 889 and the number in the non-flooded plots was 149 (Table 1). Flooding before treatment with soil fumigants and fungicides significantly reduced the phytotoxicity caused by these chemicals. Flooding was the most practical method of control because it reduced the disease, was not phytotoxic, and increased yield. If the soil had been flooded there was no advantage to treating the soil with any of the chemicals.

FIGURE 1. Plot treated with 1000 pounds per acre of calcium cyanamide 40 days before transplanting lettuce. Note enlarged, abnormally smooth leaves on head in center foreground, typical of phytotoxicity caused by calcium cyanamide (Table 2, treatment 1).

FIGURE 2A. Plot treated with 100 gallons per acre of Vapam followed by six weekly applications of 1 pound each of PCNB, 75W and ferbam, 76W (Table 2, treatment 3).

FIGURE 2B. Untreated lettuce grown in soil planted to lettuce the previous season.

Preplant Soil Treatments: Some of the lettuce grown on calcium cyanamide-treated plots on mineral non-flooded soil exhibited enlarged midribs, thick veins, and distorted leaves with abnormally smooth margins (Fig. 1). Variations included marginal necrosis, stunting, and improper heading. This phytotoxicity was of minor importance on mineral soil that had received leaching rains if the interval between application and planting was at least 2 months. The injury apparently was associated with factors in the soil that were altered by heavy rainfall or flooding. Some reduction in yield was noted on peat soil treated with 1000 pounds per acre of calcium cyanamide 1 month before transplanting. A 2-month interval between application of calcium cyanamide and planting was not sufficient to completely eliminate the phytotoxicity on mineral soils that received little or no rainfall. One thousand pounds of calcium cyanamide per acre reduced sclerotiniose and increased yield on mineral soils, but the results on peat soils were inconsistent. The data on peat soil are not presented, but the data on mineral soils are presented in Table 2.

Table 2. A partial list of treatments for control of sclerotiniose of lettuce and their effect on disease control and yield. Data collected during the winters of 1959-60 and 1960-61 from lettuce grown on mineral soil near Sanford, Florida.

| Materials | Quantity per: acre soil[a] | Quantity per: 100 gallons foliar[b] (in pounds) | % lettuce drop 1960 | % lettuce drop 1961 | Yield (in pounds) 1960 | Yield (in pounds) 1961 |
|---|---|---|---|---|---|---|
| 1. Cyanamid[c], 21% N | 1000 lb | | | | | |
| PCNB, 75W | | 1.0 | | | | |
| Ferbam, 76W | | 1.0 | 22.5 | 6.5 | 109.2 | 128.3 |
| 2. Cyanamid, 21% N | 1000 lb | | | | | |
| Ferbam, 76W | | 2.0 | | | | |
| Hydrated lime | | 2.0 | --- | 4.0 | --- | 184.40 |
| 3. Vapam | 100 gal. | | | | | |
| PCNB, 75W | | 1.0 | | | | |
| Ferbam, 76W | | 1.0 | 21.2 | --- | 127.0 | --- |
| 4. Mylone, 85W | 300 lb | | | | | |
| PCNB, 75W | | 1.0 | | | | |
| Ferbam, 76W | | 1.0 | 25.0 | --- | 113.91 | --- |
| 5. PCNB, 75W | | 1.0 | | | | |
| Ferbam, 76W | | 1.0 | 26.2 | --- | 107.0 | --- |
| 6. Deep spaded | | | | | | |
| PCNB, 75W | | 1.0 | | | | |
| Ferbam, 76W | | 1.0 | 24.2 | --- | 109.7 | --- |
| 7. PCNB, 75W | | 2.0 | | | | |
| Ferbam, 76W | | 2.0 | --- | 23.8 | --- | 131.8 |
| 8. Nabac-25W | | 0.5 | | | | |
| Ferbam, 76W | | 1.0 | --- | 21.5 | --- | 122.0 |
| 9. Ferbam, 76W | | 2.0 | | | | |
| Hydrated lime | | 2.0 | 15.0 | 24.2 | 119.3 | 145.7 |
| 10. Phybam-S, 83.5W | | 4.0 | 20.0 | --- | 97.7 | --- |
| 11. Thiram, 65W | | 2.0 | 23.7 | 24.8 | 111.2 | 163.1 |
| 12. Check, Shell D-D | | | 38.3 | 35.0 | 96.8 | 99.4 |
| 13. Untreated | | | 32.9 | --- | 94.2 | --- |
| LSD 5% | | | 14.4 | 7.2 | 22.2 | 45.4 |
| 1% | | | 19.0 | 9.7 | 29.8 | --- |

[a]Applied to the soil in one application 1 month before planting. Shell D-D (26 gallons/acre) was applied to treatment numbers 5 through 12.
[b]Applied to the plants and surrounding soil as a spray on a weekly schedule. Six applications at the rate of 100 gallons per acre per application were made in 1960 and seven in 1961.
[c]Cyanamid = calcium cyanamide.

Drenching the soil with fungicides and fumigants before planting usually offered some protection against sclerotiniose, but unless these treatments were followed by foliar applications they were of little benefit. One of these combination treatments consisted of drenching the

soil 2 weeks before planting with 75 gallons per acre of Vapam followed by 6 weekly spray applications of 1 pound each of PCNB, 75W and ferbam, 76W. This treatment was significantly better than the untreated in disease control and yield (Fig. 2A and Table 2, treatment 3).

Rotation: Untreated lettuce grown in soil planted to lettuce the previous season showed symptoms of sclerotiniose in 51.6% of the plants (Fig. 2B). Untreated lettuce grown in soil planted to cabbage the previous two seasons showed symptoms of sclerotiniose in 25.0% of the plants. Cabbage is not so susceptible to sclerotiniose as lettuce.

Deep Plowing: Turning the top 8 to 10 inches of soil upside down with shovels reduced the incidence of the disease in three out of four tests; however, the reduction was not statistically significant.

Weekly Fungicide Applications Using Low Concentrations: A number of fungicide treatments offered some protection against sclerotiniose when used on a weekly schedule. The most effective and least phytotoxic were: 1) ferbam-hydrated lime mixture (2.0 lb each per 100 gallons per acre), 2) a PCNB, 75W-ferbam mixture (1.0 lb each), 3) ferbam (2.0 lb), 4) thiram, 65W (2.0 lb), 5) PCNB, 75W (2.0 lb). Examples of these data are presented in Table 2. Tolerances have not been established for use of these chemicals on lettuce. Residue samples have been collected and approval for their use is being sought.

Two Fungicide Applications Using High Concentrations: Two applications of the fungicides following reduced incidence of the disease in most of the tests in which they were used, but the reductions were not always statistically significant, nor were they great enough to be considered as good commercial controls (Table 3). Used in conjunction with cultural methods and the more effective preplant chemical treatments previously listed they provided commercial control. These treatments are in descending order of performance: 1) PCNB, 75W-ferbam mixture (7.5 lb each per 100 gallons), 2) ferbam, 76W (15.0 lb).

Table 3. A list of treatments and their effect on sclerotiniose of lettace grown on peat soil near Zellwood, Florida during the winter of 1955-56.

| Materials and quantity per acre | Number of applications | % lettuce drop |
|---|---|---|
| 1. PCNB 75W, 30 lb | 2 | 11.7 |
| 2. PCNB 25, EC, 30 lb | 1 | 16.2 |
| 3. Vapam, 50 gal. | 1 | 24.6 |
| 4. Mylone 85W, 235 lb | 1 | 22.9 |
| 5. Allyl alcohol, 50 gal. | 1 | 25.0 |
| 6. Urea formaldehyde, 100 gal. | 1 | 22.5 |
| 7. Ferbam 76W, 30 lb | 2 | 7.9 |
| 8. Untreated | | 19.1 |
| LSD 5% | | 7.5 |
| 1% | | 10.4 |

At dosages of 15 pounds per 100 gallons per application, PCNB, 75W caused moderate injury under some environmental conditions. This injury was minimized by applying PCNB, 75W in clear weather just after mid-day. The addition of ferbam to PCNB also reduced injury caused by high dosages of PCNB, 75W. PCNB, 25E was phytotoxic under all conditions.

Preplant Soil Treatments Combined with Foliar Sprays: No single treatment consistently reduced the incidence of sclerotiniose of lettuce sufficiently to be considered as a good commercial control. A combination of preplanting soil and postplanting foliar treatments was found to be necessary to obtain a satisfactory control (Table 2).

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# THE DEVELOPMENT OF MYCOSPHAERELLA BLACK ROT AND PELLICULARIA ROLFSII ROT OF WATERMELONS AT VARIOUS TEMPERATURES

N. S. Luepschen[1]


## Summary

Watermelons inoculated with Mycosphaerella melonis and Pellicularia rolfsii were incubated at different temperatures to study the effect of temperature on these postharvest decays. Growth of the organisms in plate culture was also measured. Mycosphaerella black rot increased progressively from 45° to 75° F; at 85° the rate of decay decreased. The minimum temperature for growth of P. rolfsii was somewhat above 45° since decay did not progress at this temperature. Some growth occurred at 55° and rot was the most extensive at 90°. Mycelial growth rate in vitro paralleled decay development.


## INTRODUCTION

Incidence of black rot caused by Mycosphaerella melonis (Pass.) Chiu & J. C. Walker has been increasing as a decay of watermelons originating in the southeastern United States. Occasional losses per carload from black rot have been as high as 30 to 35%, particularly with the Charleston Gray variety, which is shipped in large volume during mid-season.

Another decay of watermelons, caused by Pellicularia rolfsii (Curzi) E. West (Sclerotium rolfsii Sacc.), has been reported recently from the Chicago market (3). This rot was observed in several carlots during the 1960 season.

The incidence of these two diseases on melons arriving in the market has apparently increased in the past 2 years. Since detailed information on M. melonis and P. rolfsii in the role of postharvest decays of watermelons is lacking, it semed desirable to investigate the temperature relationships involved. The present study was initiated to determine the approximate rates of decay on artificially inoculated watermelons held at various temperatures. A comparison of the rate of decay with growth of the pathogens in vitro was also made.

## MATERIALS AND METHODS

Charleston Gray variety watermelons were purchased on the Chicago market. Melons free from bruises, decay, and as far as possible from abrasions, were inoculated. With a sterile scalpel, a small quantity of agar with mycelium from fresh cultures of the pathogens was inserted about 1/4 inch into the rind. Six inoculations and one sterile check wound were made on each melon and the wounds were then sealed with cellophane tape. This was removed from all wounds after 3 to 5 days when lesions were usually apparent.

With M. melonis, three series of inoculated melons were used, each series consisting of one melon at each temperature, except at 40° F, where only one series was run. Melons were left at room temperature (78° ± 2°) for 16 hours before incubating at the desired temperatures. Disease development was observed at the following temperatures: 40°, 45°, 55°, 65°, 75°, and 85°. No attempt was made to control relative humidity. Lesion diameter was measured in millimeters at weekly intervals.

With P. rolfsii, four series of one melon each were held at each temperature. Melons were kept at room temperature for 24 hours after inoculation before incubating at the following temperatures: 35°, 45°, 55°, 65°, 78°, and 90° F.

In vitro studies of the growth rate of M. melonis and P. rolfsii were made simultaneously with the melon-decay tests. Potato-dextrose agar plates were inoculated with agar-mycelium discs 10 mm in diameter obtained from actively developing plate cultures. Ten Petri plates were used at each temperature. Large diameter plates (150 mm) were used for the disc cultures of P. rolfsii at the higher temperatures.

## RESULTS AND DISCUSSION

Black rot: The data for Mycosphaerella black rot are summarized in Figure 1 as the mean diameters of lesion and mycelial development. No black rot infections appeared on the water-

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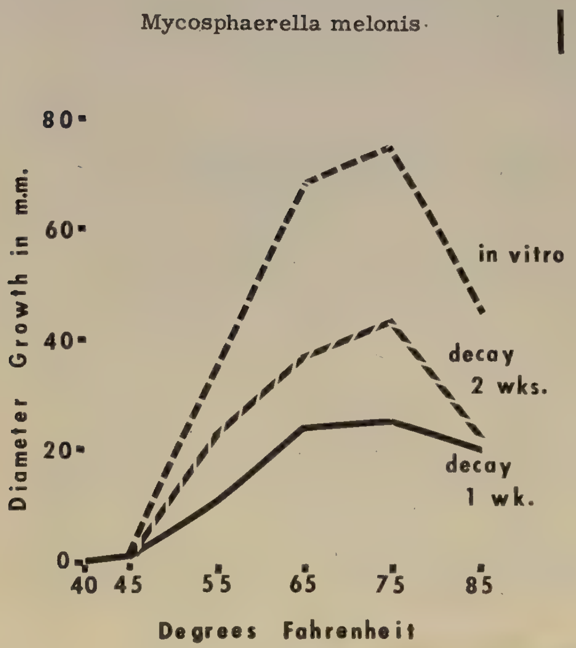


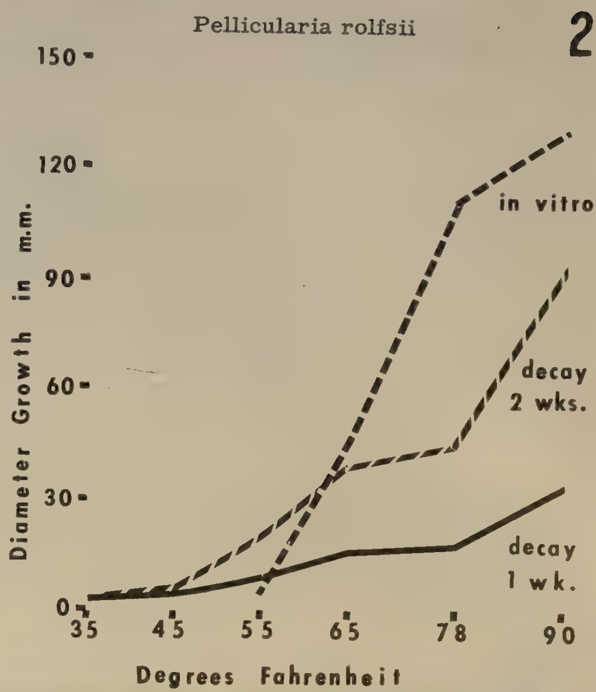


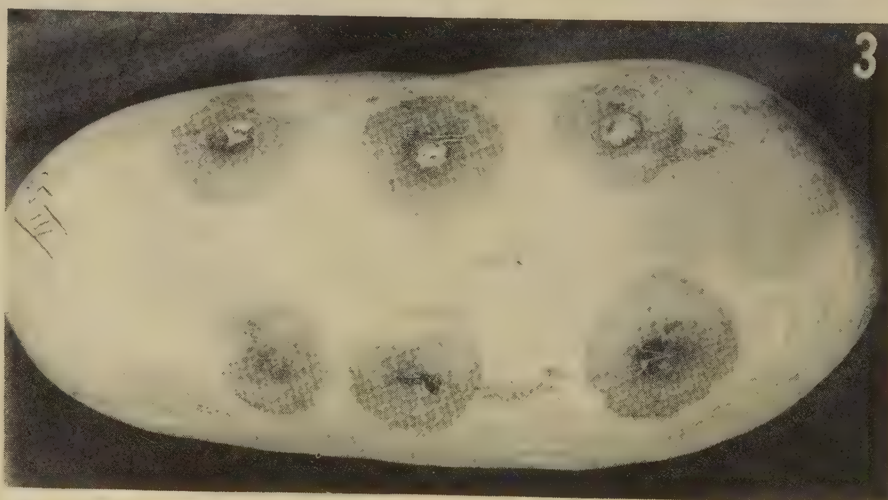


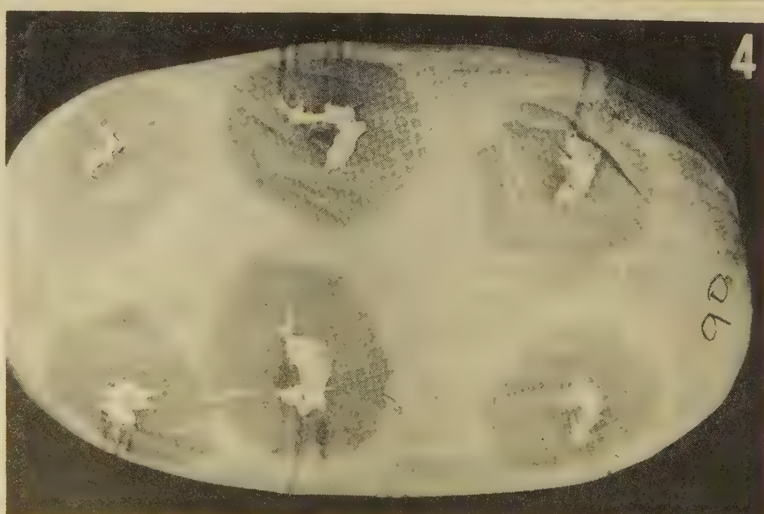


FIGURE 1. Black rot of inoculated watermelons after 1 and 2 weeks, and mycelial growth of M. melonis in vitro after 7 days at various temperatures.

FIGURE 2. Decay caused by P. rolfsii on inoculated watermelons after 1 and 2 weeks, and mycelial growth in vitro after 4 days at various temperatures.

FIGURE 3. Black rot on watermelon inoculated with M. melonis, after 15 days at 65° F.

FIGURE 4. P. rolfsii decay on inoculated watermelon after 10 days at 90° F. Wefts of mycelium and droplets of ooze have erupted out of the decayed tissues.

melon held at 40° F. Very little decay developed at 45°. At higher temperatures (Fig. 3), black rot developed progressively faster, reaching a maximum of decay extension at 75°. Between 75° and 85° there was a decrease in the rate of development. Wiant (4) obtained somewhat similar results in studies of decay of cucumber with M. melonis.

The in vitro growth curve for M. melonis after 7 days is also given in Figure 1. No growth was visible at the 40° F incubation, and only slight growth at 45°. Optimum growth occurred at approximately 75° with a diminution of growth at 85°. These results are also in close accord with in vitro results of Wiant (4). Chiu and Walker (1) reported optimum growth of M. melonis on agar plates incubated at 68° to 75° for 4 days, with minimum growth occurring slightly below 53° during the same length of time.

Pellicularia rolfsii rot: Disease development of P. rolfsii is illustrated in Figure 2 as the mean diameters of decay obtained from melons incubated for 1 and 2 weeks at the various temperatures. Limited decay occurred below 55° F. An increase in temperature accelerated decay development. At 90° (the highest temperature used in these experiments) decay had spread so rapidly in 14 days that the melons had become soft, watery, and almost completely invaded with mycelium. Figure 4 shows decay after 10 days at 90°. Rind necrosis was evident in 2 days, with amber droplets of ooze and surface mycelium evident after 4 days. At 35° to 55° the pathogen was restricted to the rind, causing shallow depressions with no oozing. The decay measured at 35° to 45° may have been due to the 24-hour postinoculation holding period prior to the low temperature incubation.

Results of the in vitro test with P. rolfsii are also shown in Figure 2. As in the decay studies, mycelial growth became more rapid as the temperature increased. These results agree with the findings of Higgins (2) who showed that the optimum temperature for growth of P. rolfsii was between 86° and 95° F, the minimum temperature for growth was 46.4°, and the maximum temperature at which growth occurred was 104°. The mycelium discs in the tests reported herein did not develop at 45°, even when held beyond 4 days.

The rate of growth of mycelium of P. rolfsii, in vitro, increased with increasing temperature at a relatively faster rate than that of M. melonis. At 90° F, the highest temperature used in these experiments, the rate of mycelial growth and decay of P. rolfsii was still increasing. On the other hand, rate of growth of M. melonis was limited at 85°.

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# SPHAEROPHRAGMIUM RUST DISCOVERED IN FLORIDA ON ALBIZIA LEBBECK (L.) BENTH.

A. P. Martinez[1]

In September 1960, C. O. Youtsey, District Inspector, Division of Plant Industry, collected a rust on *Albizia lebbeck* (L.) Benth. (*Mimosa lebbeck* L., *Acacia lebbeck* (L.) Willd.). The rust was identified as *Sphaerophragmium acaciae* (Cooke) Magnus (1, 2). It has since been reported from 21 locations in 9 counties (Fig. 1).

The fungus produces small yellowish-green chlorotic areas, 0.5 to 1 mm in diameter, on the upper surface of the leaflet. The uredia and telia are produced on the corresponding lower surface of the chlorotic spots (Fig. 2). The sori are scattered at random over the leaflets but may frequently be found arranged in concentric circles. Small brown rust spots may be found also on the seed pods. The fungus does not sporulate as abundantly on the seed pods as on the foliage.

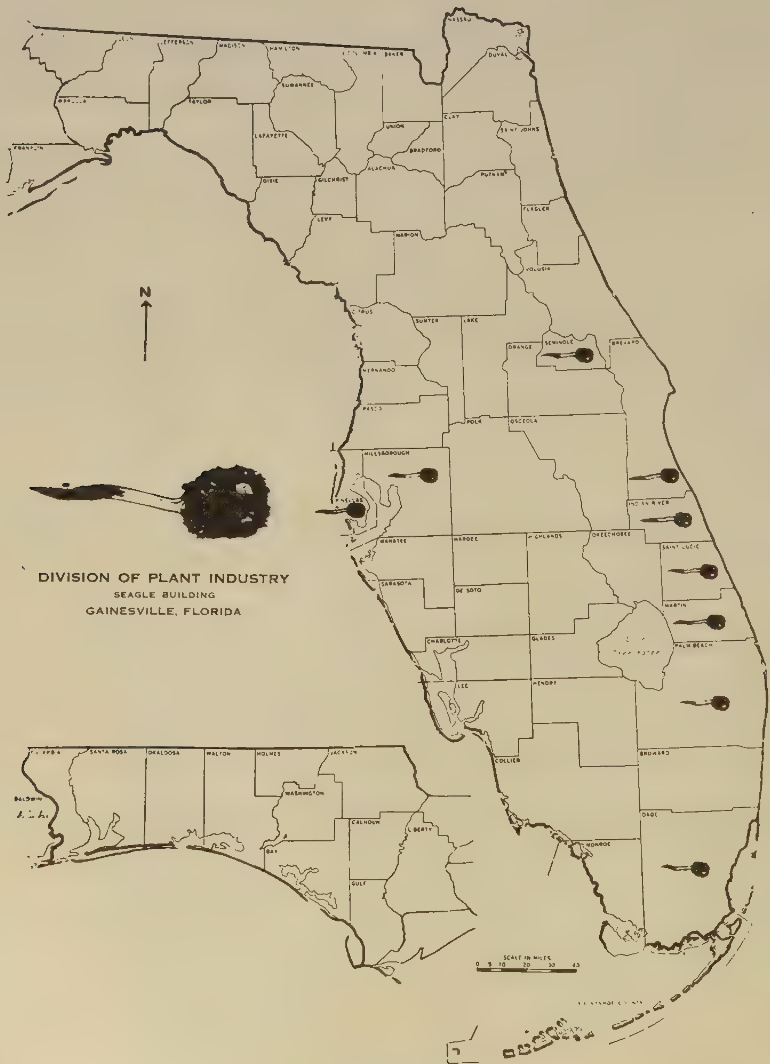


FIGURE 1. Distribution of rust in Florida during winter of 1960-1961.

[1]Plant Pathologist, Division of Plant Industry, Florida State Department of Agriculture, Gainesville, Florida. The assistance of Mr. E. West, Sr., Botanist and Mycologist, University of Florida, is gratefully acknowledged. Photographs by J. L. Messec.

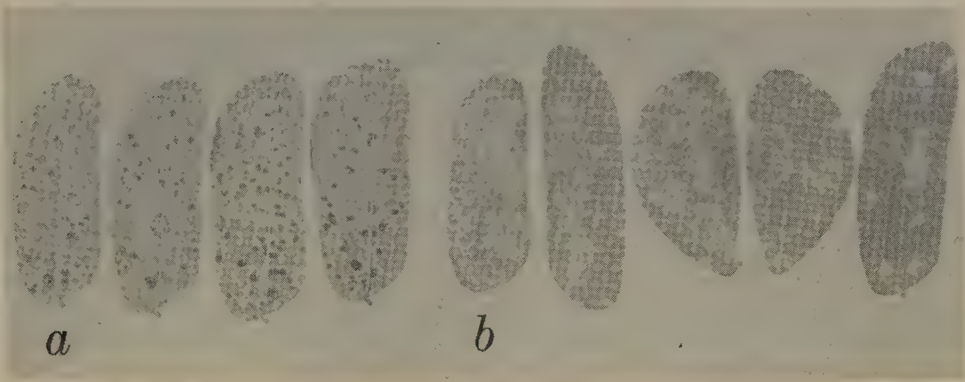


FIGURE 2. Leaflet symptoms. a -- Fruiting structures on the lower surface of leaflets. b -- Chlorotic spots on the upper surface of leaflets.

The Sphaerophragmium reported here, so far as can be determined microscopically, is the same fungus previously reported on Albizia lebbeck from other parts of the world. Herbarium specimens for comparison were graciously provided by P. L. Lentz from the National Fungus Collections, Plant Industry Station, Beltsville, Maryland.

The urediniospores (23-28$\mu$ x 20$\mu$) borne singly on short pedicels, are ellipsoid or obovoid, often reniform; wall nearly or quite colorless, echinulate, the pores usually two, and equatorial. The paraphyses are prominent but their relative position in the sorus was not verified. According to Magnus (1) these structures may form an outer cover over the spores in the early stages of development.

The teliospores appear black when examined under the low power of the dissecting microscope. Further examination under higher magnification shows the color to be light brown or tawny (Villalobos Colour Atlas OOS Line 9-10 at 7°). The cell walls, which are flat where cells join, appear darker even with transmitted light. The number of cells varies from four to eight, but undoubtedly the maximum number count will vary depending on the light because of their lattice arrangement. The ornate spiny appendages are obclavate, being thicker at the base, 2-5$\mu$, than the tapered tips which measure .6 to 1.0$\mu$ at the apex directly beneath the attached end of the recurving tips. The tips are 3-5 in number and are included in the overall length, 7-12$\mu$, of the spines. The hyaline pedicels are carrotiform, with the widest part, 9$\mu$, nearest the point of attachment. Frequently the width of the apex, 5-6$\mu$, is constant throughout the entire length, 85-94$\mu$, of the stalk. The average width of 20 spores measured is 28$\mu$, ranging from 21-43$\mu$. The spores are not as wide as they are long. Twenty-four spores measured average 39$\mu$ in length, varying from 30-60$\mu$.

So far as can be determined, this is the first report of a rust of this genus collected in Florida. Specimen material has been deposited in the Herbarium of the University of Florida, Agricultural Experiment Station, Gainesville, Florida, as Number F-46918, dated September 28, 1960.

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# AN IMPROVED METHOD FOR DETERMINING RESISTANCE TO FUSARIUM STEM ROT OF SWEETPOTATOES

G. C. Hanna, A. G. Gentile, and K. A. Kimble

## Summary

A quick method of testing sweetpotatoes for resistance to stem rot is described. The manifestation of the disease was greatly hastened by submerging the internodally-cut ends of terminal cuttings into a spore suspension and at the same time crushing them with a pair of pliers. Susceptible plants presented severe symptoms of the disease within 7 to 15 days of inoculation.

Because P.I. 153655 ("Tinian") has exhibited the highest degree of resistance of all material so far observed, it was extensively used in the breeding program for 1959-60. More than 4000 seedlings with "Tinian" parentage were tested for resistance to stem rot during this period. Several highly resistant selected lines are at the present time undergoing further study.

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Stem rot (Fusarium oxysporum f. batatas (Wr.) Snyd. & Hans.) occurs in nearly every State in which sweetpotatoes are grown. The general severity of the disease and the difficulty in controlling it emphasize the importance of the development of resistant varieties adaptable to the various growing areas.

Several eastern varieties of sweetpotatoes have been reported resistant but "not entirely immune" to the disease (1). None of the commercial varieties grown in California at the present time is resistant to stem rot. One of the objectives of the current sweetpotato breeding program at the University of California at Davis is stem rot resistance. This report describes an improved method for inoculating seedlings of sweetpotatoes with the stem rot fungus.

## MATERIALS AND METHODS

The highest degree of resistance from any source thus far tested has been observed in P.I. 153655 ("Tinian"), as reported earlier by Steinbauer (3, 4, 5).

Inoculum: Single spore culture stocks of the pathogen were used as the source of inoculum. Streak transfers were made onto potato-dextrose agar slants which were incubated from 6 to 7 days at a temperature of 25° to 27°C. Spore suspensions were then prepared by gently rubbing and washing the fungal growth on the surface of each slant with distilled water. Each slant provided 300 cc of inoculum, sufficient for the inoculation of from 40 to 50 sweetpotato terminals.

Inoculation: McClure (2) concluded that "the principal mode of infection of sweetpotato sprouts is by way of vascular wounds such as freshly cut stems, roots or fresh leaf scars. Infection does not occur through uninjured stems or roots, root eruption wounds or the callus of a healed basal wound." He successfully inoculated freshly cut sweetpotato sprouts and sprouts having freshly cut roots by dipping their cut ends into a spore suspension, and observed symptoms of the disease 2 weeks after inoculation. No references to air and soil temperatures were made in his report.

McClure's methods of inoculation were followed in the present study. The inoculated cuttings were then planted in sterile soil with a thermostatically controlled temperature of 80° F in a greenhouse with an average air temperature of 85°. These infected plants, in contrast to McClure's, did not manifest symptoms until nearly a month after inoculation. A faster method of testing sweetpotatoes for resistance to stem rot under the aforementioned environmental conditions was therefore sought. A more rapid symptom expression was achieved by means of the following modification of McClure's methods.

Succulent terminal cuttings 6 to 8 inches long from seedlings with Tinian parentage as well as terminals of a susceptible control variety were collected in the field and placed immediately in vials of water to prevent wilting. Within half an hour of collection they were inoculated by crushing the internodally-cut basal ends with flame-sterilized pliers while the stem ends were submerged in the spore suspension. With this method of inoculation, susceptible plants manifested striking symptoms of the disease 7 to 15 days after inoculation.

The symptoms observed in susceptible plants included: extensive vascular discoloration, wilting and yellowing of foliage, longitudinal internodal lesions and, in some cases, death of the plant. The seedlings were split longitudinally and rated according to the degree of vascular dis-

coloration manifested, from 0 to 4, as follows:

0 - no visible discoloration above the point of inoculation
1 - discoloration confined to the node immediately above the point of inoculation
2 - vascular discoloration extending to the lower half of the stem
3 - vascular discoloration extending to the apex of the stem
4 - plant dead

Brown discoloration and lack of callus formation were observed in all tested plants at the point of inoculation, regardless of the degree of symptom expression.

In the population of 4564 $F_1$ seedlings, 557 received 0 and 1 ratings. Of this number, 318 with desirable horticultural characteristics have been selected for further study.

No attempt was made to secure genetic data, since only plants containing a large amount of carotene were saved for further horticultural evaluation.

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# N, P, AND K IN LEAVES OF CITRUS TREES INFECTED WITH RADOPHOLUS SIMILIS[1]

A. W. Feldman, E. P. DuCharme, and R. F. Suit[2]

Abstract

Leaves from citrus trees infected with R. similis are lower in both K and N than leaves from healthy trees. Lowest K in leaves exists in the visibly declined trees but low K also exists in the first two to three trees of the apparently healthy area. There was no difference between levels of P in decline or healthy trees. Application of high levels of K in fertilizer resulted in higher K levels in healthy and decline trees. High rates of DBCP, D-D, and EDB, which destroyed 60 to 80% of the feeder roots, did not materially alter the K levels in the tree with a 3-month period.

Citrus trees parasitized by Radopholus similis (Cobb) Thorne have sparse foliage, small leaves, and greatly reduced terminal growth, so that a definite demarcation exists between the diseased and healthy area in the grove. Trees so affected give the appearance that soil fertility is lacking and that the condition might possibly be mitigated by increasing fertilizer applications. In the course of investigations on host response to nematocides and fungicides applied to the soil, there appeared to be a difference in the content of N and K in the leaves from decline and healthy trees. Consequently, a study was undertaken to ascertain the N, P, Ca, and K levels in both healthy and decline trees and further to determine whether any changes in the content of these elements might be reflected in host vigor. Such information would also be useful in diagnosing trees in the early stages of spreading decline, thus facilitating the delineation of decline areas.

## REVIEW OF LITERATURE

The effect of nematodes on the mineral content of the host has received considerable attention in the past 10 years. A lower potassium level in leaves of walnut (5) and rose (10) was found in plants attacked by Pratylenchus vulnus. Similar results were also noted with boxwood infected with Pratylenchus spp. (14). Leaves of peach seedlings infected with Meloidogyne incognita and M. javanica accumulated more potassium than noninfected plants (3). Phosphorus and nitrogen were also found to be significantly higher in roots of boxwood infected by Pratylenchus (14). Trichodorus christiei, at all levels of infection, did not affect the percent concentration of N, P, or K in tomato plants but did affect the total N, P, or K content by the reduction of tops and roots (6).

The mineral nutrition of the host plant was also observed to have a profound effect on the nematode-host relationship. Oteifa (7) and Kirkpatrick, et al. (4) found that the population of nematodes could be reduced by increasing the amount of potassium supplied to the host. Meloidogyne javanica parasitizing tomato plants deficient in a single major element (especially nitrogen) matures more quickly than when it feeds on plants receiving a full complement of nutrients (2). Oteifa (8) noted that first egg production by Meloidogyne incognita was 40, 24, and 16 days from time of inoculation in plants supplied with deficient, optimum, and excessive amounts of potassium.

## PROCEDURE

Analytical: The groves studied were in the central Florida ridge section and included oranges (vars. Pineapple, Hamlin, and Valencia) and grapefruit (var. Duncan). All trees were on rough lemon rootstock and were between 8 and 40 years old. These groves varied as to fertilizer and spray applications. One hundred leaves per tree, taken at random from the nonfruiting spring flush twigs (except where indicated), were used for analyses. Leaves were

[1]Florida Agricultural Experiment Station Journal Series No. 1272.

[2]Plant Pathologists, University of Florida Citrus Experiment Station, Lake Alfred. The authors wish to acknowledge gratefully the cooperation of Dr. R. C. J. Koo for the NPK determinations. Thanks also to Mr. J. M. McNamee of the Division of Plant Industry of the Florida State Department of Agriculture for his able assistance in part of this study.

washed, dried at 70° C for 96 hours, and then ground in a Wiley mill. Nitrogen was measured by the semi-micro Kjeldahl procedure (15) and phosphorus by the ammonium molybdate vanadate method (1). A Beckman, model DU, flame photometer with hydrogen-oxygen flame was used for the analysis of potassium and calcium.

Grove Survey: Root samples were collected in July 1959 from nine burrowing nematode infected groves showing a definite decline margin. Ten trees were sampled in each grove, beginning at the exact margin with the last tree showing visible decline symptoms and proceeding in the same row into the healthy area. In September 1959 samples were collected from five decline groves. In this series, 15 trees were sampled in each grove, again beginning with a tree showing visible decline symptoms and proceeding into the healthy area. There appeared to be no distinction as to the general care or fertilizer program for either the decline or healthy areas in each grove.

Soil Treatments: A. Fertilizer. The soil around six decline and six healthy 25 to 30 year-old orange trees (var. Pineapple) was treated in October 1959 with 8.5 pounds of KCl (equivalent to 5.1 pounds of $K_2O$) from the trunk to 5 feet beyond the drip line.

In March 1960 a similar trial was made on burrowing nematode-infected 17-year-old Valencia orange trees. The general tree vigor was poor and many dead branches were conspicuous. Fifteen pounds of 8-0-8-5 ($N$-$P_2O_5$-$K_2O$-$MgO$) fertilizer per tree was applied to all plots at the time the potash was applied. Fertilizer of the same analysis was again applied at the same rate to all plots in June.

Leaf samples from these plots were collected each month starting at the time the treatment was made.

B. Nematocides. The nematocides 1,2-dibromo-3-chloropropane (DBCP), ethylene dibromide (EDB), 1,3-dichloropropene (1,3-D), and 1,3-dichloropropene-1,2-dichloropropane (D-D) were each applied three times by sprinkle irrigation (12) for a total application of 30, 30, 45, and 60 gallons per acre respectively. Fifty-five Valencia trees, 17 years old and showing extensive decline symptoms, were used in each nematocide plot. Treatments were applied to each plot in mid-February, mid-March, and mid-April of 1960. The area was fertilized in March and in June with 15 pounds per tree per application with 8-0-8-5 fertilizer. Samples for root profile determinations were collected from cores of soil 8 inches in diameter in July from each of six trees at the 0-2, 2-4, and 4-6 foot levels. Leaves for NPK analyses were also collected at this time.

## RESULTS

Grove Survey: Root samples taken from the decline margin all contained *R. similis* and in most cases the parasites were found on the adjacent tree which did not show apparent decline symptoms.

The low level of potassium and nitrogen in the first two to three trees beginning at the exact margin with the last tree showing visible decline symptoms and proceeding into the healthy area was essentially the same for both grapefruit and orange trees (Fig. 1). The content of nitrogen and potassium was essentially the same from the fifth to the tenth tree beyond the margin (Fig. 1). Also, no important changes in the content of these elements was observed where there was a slight change in the soil elevation. The levels of nitrogen, phosphorus, and potassium from leaves collected in September tended to be somewhat lower than those collected in July (Fig. 1). Similar trends were noted by Smith and Reuther (11). There was no difference between levels of either phosphorus or calcium in decline and healthy trees.

Fertilizers: Where potassium alone was applied to the soil, potassium either was not taken up by the roots of decline trees or, if taken up, it was not moved to the leaves (Table 1). The potassium had no visible effect on the vigor of either decline or healthy trees.

In the trial where fertilizer as well as extra potassium was used, the general tree appearance of all was temporarily improved until after the fall dry season, when the trees slowly began to exhibit the typical spreading decline appearance. The potassium level increased in decline trees soon after a complete fertilizer was applied and remained essentially constant at a high level for the next 3 months (Table 2). This level was independent of additional fertilization with KCl. This increase in K is also explained, in part, by the inclusion of some leaves from the current spring flush. Such leaves normally have a higher K content. The K levels from the samples collected in July were above the optimum (Table 2) according to the Reuther and Smith standards (9). These standards apply only to 4 to 7 month-old spring flush leaves.

Nematocides: Three months following the last application of nematocides there was no correlation between the degree of root injury caused by the nematocides and the level of leaf potassium observed. This was especially noticeable with D-D, which reduced the feeder roots

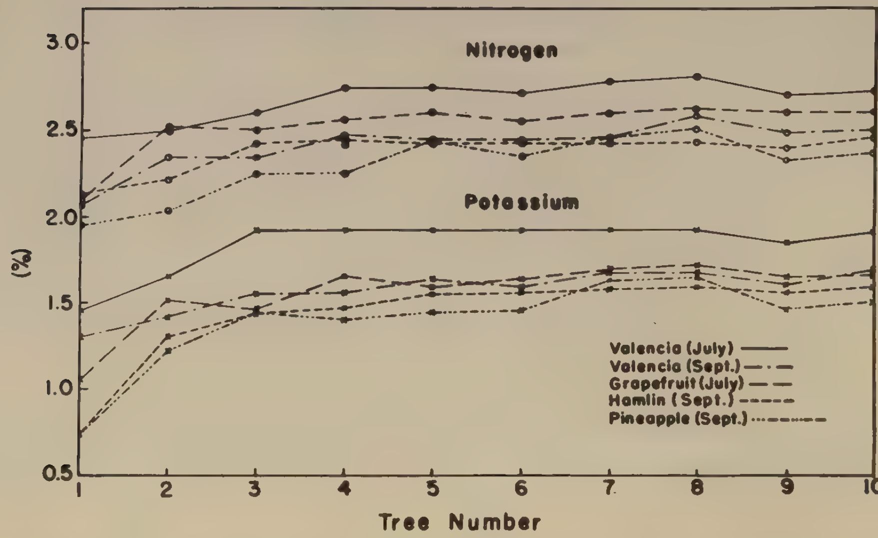


FIGURE 1. Comparison of the N and K levels in leaves of varieties of citrus. The first sample was collected at the exact margin with the last tree showing visible decline symptoms (No. 1) and proceeding in the same row into the healthy area. Data from 14 decline groves surveyed.

1. Orange (var. Valencia) sampled July (average of 5 groves).
2. Orange (var. Valencia) sampled September (average of 3 groves).
3. Grapefruit (var. Duncan) sampled July (average of 4 groves).
4. Orange (var. Hamlin) sampled September.
5. Orange (var. Pineapple) sampled September.

by approximately 60% but the level of leaf potassium was still comparable to the nontreated decline trees (Table 3). The high potassium level in the leaves of nontreated decline trees again reflects the effect of high fertilizer applications. No burrowing nematodes were found in root samples from trees in the nematocide plots in August 1960, but some burrowing nematodes were found in some samples of roots collected in January 1960 from the D-D, EDB, and 1,3-D plots.

No change in leaf nitrogen (average 2.70%) was noted in any of the nematocide plots, except in the EDB plot which was approximately 15% lower than the check.

## DISCUSSION AND CONCLUSIONS

The potassium level in leaves of citrus trees infected with R. similis is lower than in leaves of noninfected trees. Since the level of potassium in the leaves can be altered by high rates of K fertilizer applied during the time of active growth, it appears that the potassium level in leaves could not be used as a reliable diagnostic tool for ascertaining the presence of R. similis. That a sharp demarcation does not exist between R. similis infected and noninfected trees (approximately 3 or 4 trees beyond the visible margin) would also preclude the use of K levels as a possible reference for R. similis infection. Based on the Reuther and Smith standards (9), the average potassium levels of leaves obtained from 9 of the 14 decline groves surveyed may be considered to be in the optimum range, that is, 1.16 to 1.75%. Five groves had potassium levels from 0.73 to 1.10 for an average of 0.95%. These groves had apparently received a minimum of care since R. similis had been found in the groves.

The fall application of potassium failed to increase potassium in leaves in the decline trees possibly because the trees were beginning to go into dormancy. It is also quite possible that the heavy rainfall (approximately 10 inches) in October and November 1959 was sufficient to carry the soluble potassium below 24 to 30 inches where relatively few feeder roots exist in decline trees. In several instances, trees in rather poor vigor as a result of foot rot had as much potassium as the adjacent healthy trees.

Table 1. Potassium level in leaves of healthy and decline citrus trees (var. Pineapple) following application of potassium to the soil.

| Date sampled | % potassium in leaves: decline | % potassium in leaves: healthy |
|---|---|---|
| 10/15/59[a] | 1.25 | 1.75 |
| 11/13/59 | 1.25 | 2.04 |
| 12/14/59 | 1.29 | 1.88 |
| 1/11/60 | 1.19 | 1.99 |
| 7/9/60 | 1.49 | 1.71 |

[a]8.5 pounds KCl applied per tree 10/15/59.

Table 2. Potassium level in leaves of decline Valencia orange trees following high levels of applied potassium and fertilizer.

| Date sampled | % potassium in leaves: with additional potassium | % potassium in leaves: without additional potassium |
|---|---|---|
| 3/14/60[a] | 1.29 | 1.16 |
| 4/19/60 | 2.29 | 2.18 |
| 5/24/60 | 1.69 | 2.00 |
| 6/21/60[b] | 1.95 | 1.88 |
| 7/27/60 | 1.95 | 1.88 |

[a]KCl 8.5 pounds per tree plus 15 pounds 8-0-8-5 applied just after taking first leaf samples.
[b]Second application of fertilizer applied. No additional KCl added.

Table 3. Leaf potassium and root weight from decline trees sampled 3 months after last application of nematocides.

| Treatment | Rate[a] (gallons/acre) | % potassium in leaves | Dry root weight (in grams)[b] |
|---|---|---|---|
| None | -- | 2.06 | 5.0 |
| D-D | 60 | 2.10 | 1.9 |
| DBCP | 30 | 1.71 | 0.9 |
| EDB | 30 | 1.31 | 1.3 |
| 1,3-D | 45 | 1.83 | 1.6 |

[a]Nematocides applied by sprinkle irrigation. Rates indicate total amount used in three equal applications over a 60-day period.
[b]Average of six trees. Weight of roots obtained in a soil core 6 feet deep using an 8-inch auger. Samples obtained at drip line.

A tree with spreading decline has approximately half as many effective feeder roots as a healthy tree and those roots are usually in the top 30 inches of soil. Root pruning by R. similis and its subsequent effects on the host may account, in part, for the low potassium levels of decline trees. This might suggest that R. similis, even at relatively low populations, has far-reaching effects on the general host physiology since only an occasional R. similis is found on the second or third tree ahead of the visible margin. Suit and Ford (13) also noted that respiration and catalase activity of rootlets and leaves increased in rate from the visible decline area up to and including the third healthy tree beyond the decline margin. Nematocides on the other hand, which destroyed 60 to 80% of the feeder roots, did not materially alter the K levels in the trees within a 3-month period. This might suggest that there is sufficient K reserves in the tree and that destroying 60 to 80% of the feeder roots by nematocides would not necessarily cause a rapid reduction in K levels. Similarly trees would possibly have to be infected for more than a year before the K level was significantly reduced. If such is the case, then the lower K levels noted in the first and second tree beyond the visible margin might possibly indicate that the burrowing nematode had been active on these trees for more than 1 year even

though no visible decline symptoms are evident.

Trees infected with R. similis can take up nitrogen, potassium and phosphorus from the soil, though the degree of efficiency of uptake and utilization may be considerably less than in healthy trees.

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# SOIL FUNGICIDES FOR CONTROL OF PEA ROOT ROT IN GREENHOUSE TESTS[1]

John L. Lockwood

Summary

Field soil naturally infested with at least four different root pathogens of pea was treated with each of 24 fungicides at rates equivalent to 10, 30, and 100 pounds per acre in greenhouse tests. The experimental materials C-272, D-113, 6N49, 3944, 4223, and U-587 reduced disease severity at rates of 30 or 100 pounds per acre, but never gave complete control. D-113 and 6N49 gave some disease control at the 10 pounds per acre rate. In a small field test, D-113 reduced severity of root rot at the rate of 30 pounds per acre.

Root rot of pea, a complex of several fungus diseases, is prevalent in Michigan and limits yields and quality of this crop. A search for sources of resistance to *Aphanomyces euteiches* and *Fusarium solani* f. *pisi*, important components of the pea root rot complex, has revealed certain pea introductions with higher levels of resistance than commercial varieties to these two fungi (3). Breeding work is in progress using pea introductions as resistant parents (2). It was thought desirable also to examine the possibility of controlling root rot with soil fungicides. Consequently, small-scale greenhouse tests were done using 24 fungicidal materials in a naturally infested field soil.

## MATERIALS AND METHODS

The fungicides used in the tests were as follows: 20% *trans*-1,2-bis (ethylsulfonyl) ethylene (C-272), 5% 1,2 dichloro-1-(methylsulfonyl) ethylene (D-113), 20% 1,2 dichloro-1-butyl sulfonyl ethylene (D-121), 5% *trans*-1,2-bis (n-propylsulfonyl) ethylene (B-1843), 20% p-dimethylamino-benzenediazo sodium sulfonate (Dexon), 5% *p*-dimethylaminobenzenediazo sodium sulfonate, 5% pentachloronitrobenzene (Dexon-PCNB), all supplied by Chemagro Corp.; 75% pentachloronitrobenzene (PCNB) (Terraclor), 50% 2-pyridinethione 1-oxide-ferric salt (Omadine Fe), 50% 2-pyridinethione 1-oxide-zinc salt (Omadine Zn), 50% 2-pyridinethione 1-oxide-manganese salt (Omadine Mn), 50% 2-pyridinethione 1-oxide-disulfide derivative (Omadine disulfide), all supplied by Olin Mathieson Chemical Corporation; 50% *N*-trichloromethylthio phthalimide (Phaltan), and 50% *N*-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide (captan) (Orthocide 50), from California Spray-Chemical Co.; 10% 2,3 dichloro-1,4 naphthoquinone, 15% pentachloronitrobenzene (ME-7364), and (ME-5961, 5%) from Niagara Chemical Div., Food Machinery and Chemical Corp.; 5% 5-chloro-4-phenyl-1,2-dithiol-3-one (H-3944) and 5% 5-chloro-4(p-tolyl)-1,2-dithiol-3-one (H-4223), from Hercules Powder Co.; 50% polyethylene thiuram sulfides (Thioneb), from U. S. Rubber Co.; 65% tetramethylthiuram disulfide (thiram) (Thylate), from E. I. duPont de Nemours, Inc.; 20% quinoxime benzoyl hydrazone (4452) from Geary Research Lab.; 10% chlorinated heterocyclic S compound (6N49), from Diamond Alkali Co.; 0.26% cycloheximide, 15% pentachloronitrobenzene (Acti-dione RZ), from Upjohn Co.; 33% *O*, *O*, *O*-trimethyl phosphorothioate (SD-4741), from Shell Development Co.; a stabilized halogenated carbonyl compound (U-587) (100%) from Norwich Pharmical Co.

Fungicides were diluted with soil to give final concentrations of 0.1, 0.3, and 1.0 grams of active ingredient per 1/3 cubic foot of soil (approximately equivalent to 10, 30, and 100 pounds per acre). Mixing was done in a hand-turned, off-center mixing machine.

Soil used was Conover loam from East Lansing, Michigan in which peas had been grown for several successive years. Peas grown in this field had severe root rot in 1957-1960. Soil was known to contain the following root pathogens of pea: *A. euteiches*, *Rhizoctonia solani*, *F. solani* f. *pisi*, and *Thielaviopsis basicola*. The latter two fungi appeared to predominate. Treated soil was placed in metal pans 13 x 26 x 4 inches deep, which were divided by wooden barriers into six equal sections, each containing approximately 1/9 cubic foot of soil. Thirty seeds of Miragreen peas, lightly dusted with captan, were sown 1 inch deep in each section. Pans were placed in a greenhouse with an air temperature of 24° to 28° C for approximately 4 weeks, when plants were removed and the degree of root rot estimated. Soil treated with chemicals giving some disease control was resown with pea seeds 2 to 4 weeks after evaluation. Promising chemicals were also retested one or more times in new experiments. Treatments in each experiment were done in duplicate.

[1]Journal Article No. 2814 from the Michigan Agricultural Experiment Station.

A field test using Dexon and D-113 was done in naturally infested Conover loam soil. Fungicides were applied at rates of 10, 30, and 100 pounds per acre by sprinkling on the surface of plots 1 foot wide x 10 feet long, then mixing into the top 4 to 6 inches of soil with a rotary hoe. Fungicidal treatments were separated and bounded at the ends by untreated strips. Following treatment of soil, two rows of Early Perfection pea seeds were drilled into each plot at the rate of 200 pounds per acre. Treatment and planting were done on April 26, 1960. Root rot was estimated in four samples of 50 plants each taken at random across duplicate blocks 7 and 9 weeks after planting.

Degree of pea root rot in greenhouse and field tests was estimated by rating disease in foliage, roots, and epicotyls separately using a scale from 0-3 with 0 indicating no disease, and 3 maximum disease severity. Ratings for the three parts were summed giving a maximum disease index of 9.

## RESULTS

Of the 24 fungicidal materials tested only 6 reduced severity of pea root rot in greenhouse tests (Table 1). These were C-272, D-113, 6N49, H-3944, H-4223, and U-587. Reduction in disease severity at the 10 pounds per acre rate was achieved by D-113 in each of four tests and by 6N49 in two of three tests. All other fungicides failed to reduce disease severity even at the highest rate of application (100 pounds per acre). Disease was uniform and severe in all tests. Disease indices of plants in untreated plots ranged from 5.5 to 8.5 in various tests. Undusted seeds planted in untreated soil failed to emerge. Each of the six materials that were effective in initial tests gave some control at higher rates of application when seeds were replanted in previously treated soil, but all were less effective than in initial tests. Of all fungicides tested, D-113 gave greatest control at lowest concentrations, both in initially treated soils and in replanted soils.

Table 1. Results obtained with fungicides that reduced pea root rot in naturally infested field soil in greenhouse tests.

| Material | Rate (lbs/acre) | Average disease index[a] initial test | Average disease index[a] replant test | Material | Rate (lbs/acre) | Average disease index[a] initial test | Average disease index[a] replant test |
|---|---|---|---|---|---|---|---|
| C-272 | 0 | 8.3 | 7.8 | H-3944 | 0 | 6.7 | 8.1 |
| | 10 | 8.3 | 7.8 | | 10 | 6.2 | 7.2 |
| | 30 | 5.4 | 6.3 | | 30 | 4.9 | 5.5 |
| | 100 | 5.2 | 5.7 | | 100 | 3.9 | 5.8 |
| D-113 | 0 | 7.3 | 7.9 | H-4223 | 0 | 5.8 | 8.3 |
| | 10 | 4.4 | 7.2 | | 10 | 6.0 | 8.5 |
| | 30 | 3.6[b] | 4.6 | | 30 | 3.7 | 7.5 |
| | 100 | --[c] | 3.4 | | 100 | 3.3 | 4.0 |
| 6N49 | 0 | 6.7 | 8.1 | U-587 | 0 | 5.8 | 8.3 |
| | 10 | 6.1 | 8.1 | | 10 | 5.9 | 7.8 |
| | 30 | 2.8 | 5.6 | | 30 | 4.9 | 7.0 |
| | 100 | 2.5[b] | 6.2 | | 100 | 2.8 | 5.0 |

[a]Mean of 2 to 4 tests, each done in duplicate and at different times. Disease index was based on a scale of increasing disease severity from 0-9. Pea seeds were sown immediately after soil was treated, then on completion of the test, seeds were again sown in the same soil.
[b]Delayed germination.
[c]No emergence.

Several of the materials were toxic to pea seedlings. D-113 completely prevented emergence of seedlings at the 100 pounds per acre rate in some tests, and stunted plants severely in others. Slight stunting with this compound also occurred at the 30 pounds per acre rate. Omadine disulfide, 6N49, and Dexon-PCNB delayed germination, stunted plants, and reduced stands at the 100 pounds per acre rate, but not at lower levels. On replanting in previously treated soil none of the materials was toxic.

Results of the field test, after 7 and 9 weeks, showed that D-113 reduced severity of root rot in plots treated at the rate of 30 pounds per acre but not at the rate of 10 pounds per acre (Table 2). When applied at the rate of 100 pounds per acre, D-113 prevented emergence of seedlings. Dexon gave no control.

Table 2. Effect of D-113 and Dexon on pea root rot in field tests.

| Weeks after planting | Rate (pounds/acre) | Disease index[a] D-113 | Dexon | no treatment | LSD 5% |
|---|---|---|---|---|---|
| 7 | 10 | 2.8 | 3.5 | 3.5 | 0.9 |
| | 30 | 2.0 | 3.5 | | |
| | 100 | --[b] | 3.5 | | |
| 9 | 10 | 4.9 | 5.0 | 5.0 | 0.9 |
| | 30 | 3.9 | 5.0 | | |
| | 100 | --[b] | 5.0 | | |

[a]Disease index was based on a scale of increasing disease severity from 0-9.
[b]No emergence.

## DISCUSSION

Although root rot was reduced by a few of the 24 materials tested, high concentrations were required in most cases, and complete control was never obtained with any material. Since effectiveness of the fungicides decreased with time, any benefit would almost certainly be of short duration, and probably would be reduced toward the end of the season, when root rot is often most severe. Although economics probably would not permit use of soil fungicides for control of pea root rot at present, the six materials that provided a degree of disease control in the present work merit field testing. Limited testing of D-113 in the field indicated that some reduction in root rot could be expected.

Pea root rot is a disease complex and in many situations may be due to the combined pathogenesis of *A. euteiches*, *F. solani* f. *pisi* and perhaps other fungi. This complicates control. For example, Dexon is reported to be highly effective against Phycomycetous pathogens of seedlings (1) and roots (4), including *Aphanomyces cochlioides* a pathogen of sugar beets (1), yet this fungicide failed to reduce pea root rot in the present tests where *A. euteiches* was known to be present. This probably means that *A. euteiches* was not the predominant pathogen under conditions of these tests. The effective use of chemicals (and other measures) for control of root rot of pea will require knowledge of the populations of the potential pathogens in various localities, or even in individual fields. Without this knowledge the use of soil fungicides for pea root rot control will likely give disappointing results.

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# RHIZOCTONIA CROWN ROT OF BIRDSFOOT TREFOIL (LOTUS CORNICULATUS)[1]

Charles R. Drake[2]


## Summary

Rhizoctonia crown rot (Rhizoctonia solani Kuehn) was found attacking birdsfoot trefoil (Lotus corniculatus) for the first time at Blacksburg, Virginia during July and August 1960. Damage seemed to be accentuated by the extensive prolonged drought of the 1960 growing season. Infected plants were completely dead within 2 to 4 days after the first visible symptoms appeared. Isolations from the crown tissue of the infected plants yielded typical cultures of R. solani.

Free-hand sections from the infected crown region revealed that the barrel-like hyphae of R. solani had penetrated the xylem, xylem parenchyma, and cortical tissue of the crown. Macroscopic and microscopic examination of the infected region indicated that the fungus had entered the crown through the cut stem ends.


## INTRODUCTION

Rhizoctonia crown rot (Rhizoctonia solani Kuehn) was found attacking birdsfoot trefoil (Lotus corniculatus) for the first time at Blacksburg, Virginia during July and August 1960. The disease seemed to be directly related to or was enhanced by the extensive prolonged drought of the 1960 growing season. It occurred only during the peak of the drought period and no symptoms were observed 10 days after a 1.5 inch rainfall August 22.

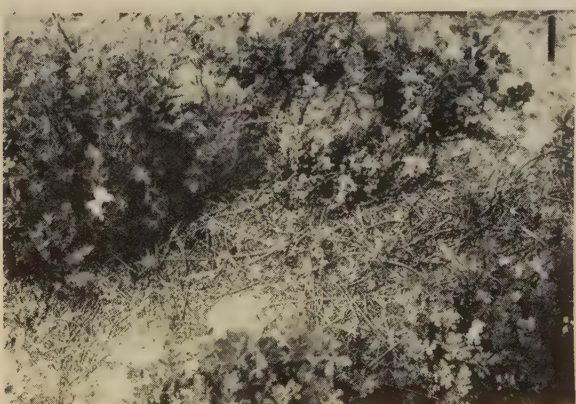


FIGURE 1. Trefoil plant killed by Rhizoc tonia crown rot.


FIGURE 2. A portion of an infected trefoil root killed by Rhizoctonia crown rot showing the characteristic consecutive, convex, narrow, brown rings in thé light gray lesion of the woody surface of the infected crown region.

[1]Cooperative investigation of Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Virginia Agricultural Experiment Station. Published with the approval of the Director of the Virginia Agricultural Experiment Station.

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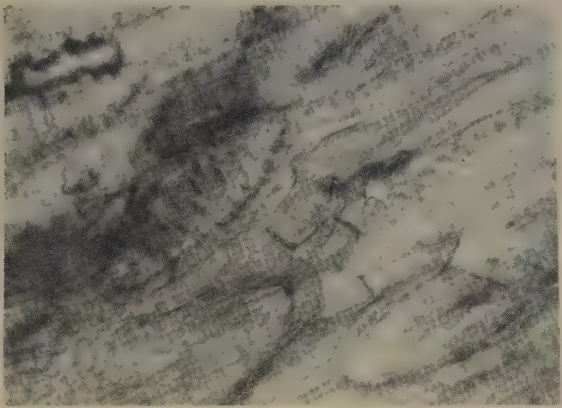

FIGURE 3. Infected crown tissue of trefoil showing hyphae of R. solani in the xylem vessels. Approx. 727 X

FIGURE 4. Trefoil root showing Rhizoctonia crown rot. Note the dark area of the cut stem where the fungus moved through into the crown region.

## SYMPTOMS

The first symptom of the disease was incipient wilting of infected plants. At that time, the foliage was lighter green than that of healthy plants. Within 24 hours, the light green foliage color changed to grayish green then to tan as permanent wilt occurred. Infected plants were completely dead within 2 to 4 days after the first visible symptoms appeared (Fig. 1).

Frequently the disease could be distinguished by certain crown symptoms. The fungus usually caused narrow, consecutive, dark brown, convex to concave rings in the light, grayish lesion zone both inside the bark and on the woody tissue of affected crowns (Fig. 2).

Infected crown tissue plated on potato-dextrose agar yielded typical cultures of R. solani. The cultures were compared morphologically with 10 different isolates of Rhizoctonia that cause foliar blight. All the crown rot isolates resembled Rhizoctonia foliar blight isolates both in rapidity of growth and in size of sclerotia produced. Further, when the crown rot isolate was used to inoculate trefoil plants in a greenhouse moist chamber, typical foliage blight occurred. Hence, the crown rot organism was identified as another isolate of R. solani.

## PATHOLOGICAL HISTOLOGY

Free-hand sections of the infected crown region were made to determine the exact location of the pathogen in the host tissue. Before sectioning the crown tissue was boiled 5 minutes in a saturated aqueous solution of chloral hydrate containing 0.01% acid fuchsin. The fungus stained a deeper red than the surrounding crown tissue and could be easily distinguished. The barrel-like hyphae of R. solani were found in the xylem, xylem parenchyma, and cortex. The hyphae did not stay in a particular xylem vessel but moved freely through the xylem walls from one vessel to another (Fig. 3). Macroscopic and microscopic examinations of the infected region indicated that the fungus had entered the crown region through the cut stem ends and moved downward and outward into the crown (Fig. 4).

## CONCLUSIONS

Crown infection of birdsfoot trefoil by Rhizoctonia solani is far more lethal than foliar blight. Because of the extreme environmental conditions required for Rhizoctonia crown rot to express itself, only minor problems are anticipated from this phase of the disease.

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# A NONPATHOGENIC WILT AND ROOT ROT OF PEAS FAVORED BY LOW LIGHT INTENSITY[1]

Beyoung H. Kwack, Stuart Dunn and Avery E. Rich[2]

Little Marvel peas (Pisum sativum) were sown in vermiculite in plastic containers. After germination the seedlings were placed under fluorescent lights of varying quality and intensity in a controlled temperature room without natural light (1). The lights were operated for 16 hours each day, alternating with 8 hours of darkness. Temperatures of 21° C during the photoperiods and 16° during the dark periods were maintained. The plants were watered twice weekly with a balanced nutrient solution (2).

The quality of light was varied by using various colored fluorescent lamps, including warm white, cool white, pink, red, yellow, blue, and green (1). The light intensity was adjusted by varying the number of lamps in each luminaire and by varying the distance of the luminaire above the pea plants. The luminaires used were 8 feet long and held up to six lamps each. They were suspended over the pea plants by means of ropes and pulleys. By slanting the position of the luminaire a continuous variation from high light intensity to low light intensity for each light quality was obtained (Fig. 1). For example, the VHO[3] cool white lamps, which produced the highest light intensity, varied from 510 to 3500 foot candles, and the GE[4] red lamps, which produced the lowest light intensity, varied from 46 to 197 foot candles (2). These were the light intensities corrected for the standard luminosity curve.

When some of the pea plants were several inches tall, the lower leaves of some became pale; then the plants gradually wilted and died. A macroscopic examination of the lower portion of the stems revealed a brown, cortical rot. The roots were poorly developed and partially decayed (Fig. 2).

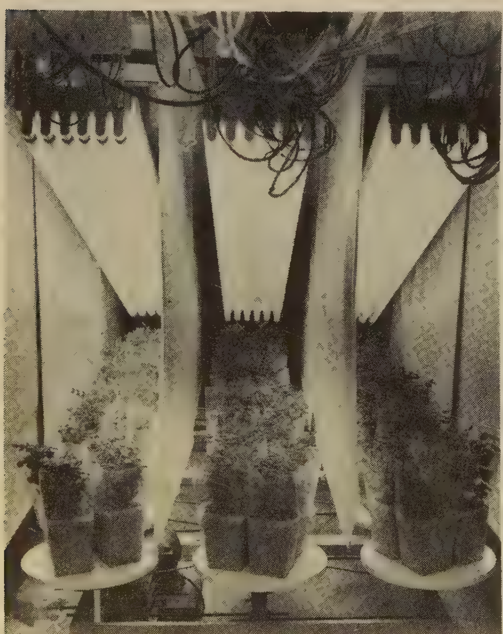

FIGURE 1. Shows position of luminaires over pea plants to provide differences in light intensity.

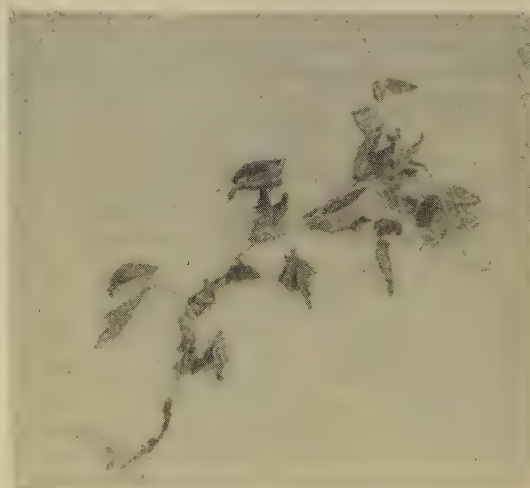

FIGURE 2. Pea plant showing typical injury to lower leaves, lower portion of stem, and roots, when grown under red lights at low intensity.

[1] Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 269.

[2] Formerly Research Assistant, Plant Physiologist, and Plant Pathologist, respectively, New Hampshire Agricultural Experiment Station.

[3] Very High Output Sylvania fluorescent lamp.

[4] General Electric T-8 Slimline fluorescent lamp.

The symptoms closely resembled the macroscopic symptoms of Fusarium root rot (Fusarium solani (Mart.) Appel & Wr. f. pisi (F. R. Jones) Snyd. & Hans.) (3).

Symptoms occurred only on plants grown under low light intensity. They were most severe on the plants grown under red light and the greatest distance from the source of the red light (46 foot candles).

It was assumed that the low light intensity predisposed the plants to attack by a pathogen which might be seedborne. Repeated attempts were made to isolate a fungus or bacterium from the affected stems and roots with the intent of testing its pathogenicity, but these organs proved to be sterile. Therefore, apparently this physiological disorder is favored by low light intensity. Also, the quality of light emitted by the red lamps may further favor its development. The lowest yields of peas were produced by plants grown under red lights at very low intensities.

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## SHORTLEAF PINE, A NEW HOST FOR COMANDRA BLISTER RUST

F. H. Berry, N. F. Rogers[1] and O. J. Dooling[2]

Commandra blister rust (Cronartium comandrae Peck) has recently been found on planted shortleaf pine (Pinus echinata) on the Clark National Forest near Salem, Dent County, Missouri. This disease of hard pines has been reported previously on knobcone, Jeffrey, lodgepole, ponderosa, and Arizona pines in the West, and pitch, Table-Mountain, jack, loblolly, Austrian, Scotch, maritime, and red pines in the East. However, this is belived to be the first report of its occurrence on shortleaf pine.

The infected trees were found among those planted on a 5-acre area being converted from oak-hickory to pine. In June 1955, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) or 2-(2,4,5-trichlorophenoxy)propionic acid (silvex) was sprayed on hardwood foliage from an airplane. In the springs of 1956 and 1957, 1-0 shortleaf pine seedlings were planted, about 1000 trees per acre. Several diseased and dead shortleaf pines with slight, spindle-shaped swellings along the stem were noticed in March 1961. Laboratory examination revealed typical pyriform aeciospores of the Comandra blister rust fungus.

Another field examination in April 1961 revealed that a number of pines throughout the plantation had either been killed or were infected with the rust fungus. The infected and dead pines averaged about 4 feet high and 1/2 inch in diameter. On one of the infected trees aecial blisters were noted on the side branches as well as along the main stem. The peridia of some of the aecial blisters were ruptured and aeciospores were being liberated.

The alternate host of this blister rust fungus is bastard toadflax (Comandra sp.). Although one species of Comandra, C. richardsiana Fernald, is widely distributed throughout Missouri, it is locally restricted to relatively small areas.

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## RICE BLAST IN EL SALVADOR

Oscar Ancalmo and Armando Alas López[1]

Piricularia oryzae Cav., the causal agent of rice blast, was first reported in El Salvador by Crandall, et al. (2) in 1951.

In 1960 an epiphytotic of the disease occurred in the rice-growing areas of the country, causing severe losses in plantings of Gulf Rose (CI-9416), a variety that is listed as resistant to blast (1). The susceptibility of Gulf Rose in El Salvador is a positive indication of the existence of physiological races of P. oryzae. The criollo variety "Cebada" was also severely affected by the disease.

Symptoms of the disease were observed on the leaves, culms, and branches of the panicle. Lesions on the neck of the culm and on the panicle branches near the base of the panicle prevented kernel filling. Lesions on the leaves were numerous and conspicuous. The Gulf Rose variety was introduced to this country in 1959 because of its resistance to "hoja blanca," a virus disease.

A cooperative project, by the Dirección General de Investigaciones Agronómicas of El Salvador with the United States Department of Agriculture, is in progress to find both blast and "hoja blanca" resistant material.

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# ARROWWEED, PLUCHEA SERICEA, ON THE COLORADO RIVER IS A HOST FOR ROOT-KNOT NEMATODES

Ivan J. Thomason and S. D. Van Gundy[1]

Soil samples obtained from a sand bar in the Colorado River at Blythe, California in October 1960 contained root-knot nematode larvae. Tomatoes planted in this soil became infected by the root-knot nematode, *Meloidogyne javanica*. In January 1961 roots of a number of plants growing on the sand bar were examined for the presence of root-knot nematodes. The roots of arrowweed, *Pluchea sericea*, were found to be infected with *M. javanica*. Relatively small galls were produced on this plant, but egg masses were present. Bessey[2] reported *Pluchea purpurascens* as a host of root-knot nematode in 1911. No other report of root-knot nematode infecting members of the genus *Pluchea* have been seen.

In March 1961 roots of arrowweed growing at the water's edge of the Colorado River several miles upstream from the original location were found to be infected with *Meloidogyne incognita*. Again small galls were produced, but large egg masses were present.

These two *Meloidogyne* spp. found on arrowweed are among the most important plant-parasitic nematodes on crop plants in California. The Colorado River is the source of most of the irrigation water for the Palo Verde, Imperial, and Coachella Valleys. Whether viable root-knot nematode larvae or eggs actually enter the irrigation systems is not known.

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